Supplemental Information

C/EBP-β-mediated upregulation of Cav1.2 calcium channel increases arterial constriction in mice with ibrutinib-associated hypertension

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Supplemental Table 1. Clinical characteristics of CLL patients with or without ibrutinib before and after propensity matching[a]

Variables	Unmatched (complete) dataset			Propensity score-matched (1:1) dataset[b]		
	Treated with ibrutinib (n=156)	Watch-and-wait (n=69)	*P* value	Treated with ibrutinib (n=37)	Watch-and-wait (n=37)	*P* value
Sex, n (%)			**<0.001**			0.626
Male	119 (76.3)	35 (50.7)		23 (62.16)	25 (67.57)	
Female	37 (23.7)	34 (49.3)		14 (37.84)	12 (32.43)	
Age, years	59.36±1.02	59 (55, 65)	0.727	57 (52, 63)	59.41±1.65	0.247
Binet stage			**<0.001**			1.000
A	13 (8.3)	20 (29.0)		3 (8.11)	4 (10.81)	
B/C	143 (91.7)	49 (71.0)		34 (91.89)	33 (89.19)	
Rai stage			**<0.001**			0.162
0-II	52 (33.3)	47 (68.1)		17 (45.95)	23 (62.16)	
III/IV	104 (66.7)	22 (31.9)		20 (54.05)	14 (37.84)	
ECOG PS			**0.005**			1.000
0-1	120 (76.9)	64 (92.8)		34 (91.89)	33 (89.19)	
>1	36 (23.1)	5 (7.2)		3 (8.11)	4 (10.81)	
BMI, kg/m^2	23.43 (21.10, 25.29)	24.22±0.40	0.105	24.01±0.49	24.06±0.48	0.944
Smoking history, n (%)	30 (19.2)	15 (21.7)	0.664	9 (24.32)	9 (24.32)	1.000
Alcohol use history, n (%)	15 (9.6)	12 (17.4)	0.098	3 (8.11)	4 (10.81)	1.000
Past medical history, n (%)						
Hypertension	40 (25.6)	18 (26.1)	0.944	8 (21.62)	10 (27.02)	0.588
Coronary heart disease	7 (4.5)	3 (4.3)	1.000	1 (2.70)	3 (8.11)	0.615
Diabetes	19 (12.2)	7 (10.1)	0.660	2 (5.41)	2 (5.41)	1.000
Renal dysfunction	5 (3.2)	0 (0.0)	0.327	0	0	
Family history of HTN, n (%)	8 (5.1)	2 (2.9)	0.727	0	2 (5.41)	0.493
ALC ($\times 10^9$ cells/L)	45.16 (8.74, 95.70)	16.35 (8.66, 39.43)	**0.005**	11.80 (3.10, 92.73)	21.02 (8.56, 64.31)	0.168
Hb (g/L)	110 (93, 125.25)	123.65±2.31	**<0.001**	115.81±3.83	122.08±3.29	0.218

PLT ($\times 10^9$ cells/L)	96 (70, 139)	156.97±6.92	**<0.001**	144.54±9.52	143.49±8.48	0.934
TC (mmol/L)	3.71 (3.13, 4.39)	3.97 (3.29, 4.70)	0.105	4.18 (3.35, 5.23)	3.93 (3.27, 4.80)	0.405
HDL-C (mmol/L)	0.98 (0.80, 1.14)	1.00 (0.85, 1.29)	0.542	1.08±0.05	0.97 (0.85, 1.22)	0.646
LDL-C (mmol/L)	2.27 (1.83, 2.79)	2.41 (1.92, 2.92)	0.157	2.67±0.15	2.51±0.12	0.415
TG (mmol/L)	1.19 (0.87, 1.54)	1.39 (0.96, 2.28)	0.091	1.24 (1.09, 1.65)	1.30 (0.96,1.93)	0.884
eGFR (mL/min)	91.30±2.24	107.95±3.62	**<0.001**	100.06 (87.80, 116.73)	96.26±4.59	0.259
Patients with abnormal HTN, n (%)	42 (26.92)	4 (5.80)	**<0.001**	9 (24.32)	2 (5.41)	**0.022**
New-onset hypertension, n (%)	29 (25.0)	1 (2.0)	**<0.001**	6 (20.69)	0	**0.024**
Worsened hypertension, n (%)	13 (32.5)	3 (16.67)	**0.342**	3 (37.5)	2 (20.0)	0.608

[a]Continuous data conforming to a normal distribution are presented as mean ± standard error of mean (SEM), and comparisons between groups were performed using the independent samples *t*-test. Continuous data not conforming to a normal distribution are described using median and interquartile range (IQR), and comparisons between groups were made using the Mann-Whitney U test. For variables with missing values, linear models were fitted across 20 multiply imputed datasets (MICE with predictive mean matching), and *P* values were pooled by Rubin's rules. Categorical data are presented as n (%), and between-group comparisons were analyzed using the chi-square (χ^2) test or the Fisher exact test.

[b]Propensity score matched (1:1) analyses were performed with a small caliper of 0.06 (0.2 of the standard deviation of the logit of the corrected propensity score) for matching potential confounders including sex, age, Binet stage, Rai stage, ECOG PS, BMI, smoking history, alcohol use history, past medical history, family history of hypertension, ALC, Hb, PLT, TC, HDL-C, LDL-C, TG and eGFR.

Abbreviations: ALC, absolute lymphocytic count; BMI, body mass index; CLL, chronic lymphocytic leukemia; ECOG PS, eastern cooperative oncology group performance status; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HDL-C, high density lipoprotein cholesterol; HTN, hypertension; LDL-C, low density lipoprotein cholesterol; PLT, platelet; PS, performance status; TC, total cholesterol; TG, triglyceride.

Supplemental Table 2. Comparative clinical characteristics in CLL patients: new-onset/worsened hypertension versus normotensive or controlled hypertension before and after propensity matching[a]

Variables	Unmatched (complete) dataset			Propensity score-matched (1:1) dataset[b]		
	Patients with new-onset or worsened hypertension (n=46)	Normotensive or controlled hypertensive patients (n=179)	*P* value	Patients with new-onset or worsened hypertension (n=11)	Normotensive or controlled hypertensive patients (n=63)	*P* value
Sex, n (%)			0.211			1.000
Male	35 (76.09)	119 (66.48)		7 (63.64)	41 (65.08)	
Female	11 (23.91)	60 (33.52)		4 (36.36)	22 (34.92)	
Age, years	63.46±1.226	58 (53.25, 65)	**0.020**	59.73±2.59	58 (54, 63)	0.732
Binet stage			0.414			0.277
A	5 (10.87)	28 (15.64)		2 (18.18)	5 (7.94)	
B/C	41 (89.13)	151 (84.36)		9 (81.82)	58 (92.06)	
Rai stage			0.281			0.178
0-II	17 (36.96)	82 (45.81)		8 (72.73)	32 (50.79)	
III/IV	29 (63.04)	97 (54.19)		3 (27.27)	31 (49.21)	
ECOG PS			0.262			1.000
0-1	35 (76.09)	149 (83.24)		10 (90.91)	57 (90.48)	
＞1	11 (23.91)	30 (16.76)		1 (9.09)	6 (9.52)	
BMI, kg/m^2	24.35±0.50	23.32 (21.00, 25.01)	**0.044**	24.68±1.08	23.93±0.36	0.439
Smoking history, n (%)	7 (15.22)	38 (21.23)	0.363	2 (18.18)	16 (25.40)	1.000
Alcohol use history, n (%)	4 (8.70)	23 (12.85)	0.439	2 (18.18)	5 (7.94)	0.277
Past medical history, n (%)						
Hypertension	16 (34.78)	42 (23.46)	0.117	5 (45.45)	13 (20.63)	0.122
Coronary heart disease	3 (6.52)	7 (3.91)	0.431	1 (9.09)	3 (4.76)	0.482
Diabetes	7 (15.22)	19 (10.61)	0.384	1 (9.09)	3 (4.76)	0.482
Renal dysfunction	0	5 (2.79)	0.586	0	0	
Family history of hypertension	2 (4.34)	8 (4.47)	1.000	0	2 (3.17)	1.000
ALC ($\times 10^9$ cells/L)	46.98 (6.93, 90.49)	23.55 (8.83, 73.68)	0.479	4.09 (1.62, 70.93)	19.30 (7.42, 83.60)	0.099

Hb (g/L)	118 (90, 128.50)	119 (99, 128.75)	0.096	119±6.87	118.94±2.75	0.993
PLT ($\times 10^9$ cells/L)	113.81±6.918	123 (77.25, 171)	0.110	132.27±8.03	146.06±7.29	0.213
TC (mmol/L)	3.80±0.12	3.85 (3.23, 4.67)	0.520	4.14±0.27	3.97 (3.29, 5.02)	0.994
HDL-C (mmol/L)	0.98±0.04	0.99 (0.82, 1.19)	0.771	1.05±0.08	0.99 (0.85, 1.25)	0.790
LDL-C (mmol/L)	2.33±0.11	2.30 (1.88, 2.82)	0.716	2.47±0.26	2.45 (2.06, 3.05)	0.915
TG (mmol/L)	1.23 (1.00, 1.69)	1.24 (0.90, 1.64)	0.948	1.38 (1.23,1.69)	1.22 (0.97, 1.80)	0.093
eGFR (mL/min)	90.78±4.45	98.52±2.23	0.107	101.15±6.88	100.03±3.31	0.895
Ibrutinib treatment, n (%)	42 (91.30)	114 (63.68)	**<0.001**	9 (81.82)	28 (44.44)	**0.022**

[a]Continuous data conforming to a normal distribution are presented as mean ± standard error of mean (SEM), and comparisons between groups were performed using the independent samples t-test. Continuous data not conforming to a normal distribution are described using median and interquartile range (IQR), and comparisons between groups were made using the Mann-Whitney U test. For variables with missing values, linear models were fitted across 20 multiply imputed datasets (MICE with predictive mean matching), and *P* values were pooled by Rubin's rules. Categorical data are presented as n (%), and between-group comparisons were analyzed using the chi-square (χ^2) test or the Fisher exact test.

[b]Propensity score matched (1:1) analyses were performed with a small caliper of 0.06 (0.2 of the standard deviation of the logit of the corrected propensity score) for matching potential confounders including sex, age, Binet stage, Rai stage, ECOG PS, BMI, smoking history, alcohol use history, past medical history, family history of hypertension, ALC, Hb, PLT, TC, HDL-C, LDL-C, TG, eGFR.

Abbreviations: ALC, absolute lymphocytic count; BMI, body mass index; CLL, chronic lymphocytic leukemia; ECOG PS, eastern cooperative oncology group performance status; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HDL-C, high density lipoprotein cholesterol; HTN, hypertension; LDL-C, low density lipoprotein cholesterol; PLT, platelet; PS, performance status; TC, total cholesterol; TG, triglyceride.

Supplemental Table 3. Univariable and multivariable logistic regression analyses of risk factors associated with new-onset/worsened hypertension in CLL patients[a]

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Sex, male	1.604 (0.758, 3.394)	0.215	1.202 (0.505, 2.864)	0.676
Age, years	1.040 (1.006, 1.076)	**0.022**	1.039 (1.000, 1.079)	0.052
Binet stage, B/C	1.521 (0.549, 4.207)	0.418		
Rai stage, III/IV	1.442 (0.737, 2.820)	0.283		
ECOG PS, >1	1.561 (0.710, 3.430)	0.266		
BMI	1.110 (1.001, 1.230)	**0.048**	1.150 (1.019, 1.298)	**0.024**
Smoking history	0.666 (0.275, 1.615)	0.367	0.697 (0.222, 2.187)	0.534
Alcohol use history	0.646 (0.211, 1.982)	0.443	0.984 (0.229, 4.233)	0.983
Past medical history				
Hypertension history	1.740 (0.862, 3.511)	0.122	1.207 (0.507, 2.876)	0.670
Coronary heart disease	1.714 (0.422, 6.958)	0.449	1.200 (0.234, 6.138)	0.826
Diabetes	1.511 (0.591, 3.868)	0.387	1.225 (0.404, 3.716)	0.719
Family history of hypertension	0.972 (0.197, 4.780)	0.972	1.071 (0.194, 5.912)	0.937
ALC ($\times 10^9$ cells/L)	1.001 (0.997, 1.006)	0.479		
Hb (g/L)	0.988 (0.975, 1.002)	0.099		
PLT ($\times 10^9$ cells/L)	0.995 (0.990, 1.001)	0.113		
TC (mmol/L)	0.888 (0.619, 1.275)	0.518	0.832 (0.314, 2.207)	0.710
HDL-C (mmol/L)	0.676 (0.217, 2.103)	0.496		
LDL-C (mmol/L)	0.920 (0.588, 1.439)	0.714	1.143 (0.359, 3.644)	0.820
TG (mmol/L)	1.014 (0.660, 1.558)	0.949	0.999 (0.500, 1.999)	0.999
eGFR (mL/min)	0.990 (0.978, 1.002)	0.109	1.001 (0.986, 1.016)	0.874
Ibrutinib treatment	5.987 (2.042, 17.555)	**0.001**	6.976 (2.140, 22.745)	**0.001**

[a]Univariable and multivariable logistic regression models were fitted in each multiply imputed dataset; the multivariable model was adjusted for age, BMI, smoking history, alcohol use, medical history, hypertension family history, TC, LDL-C, TG and eGFR.

Abbreviations: ALC, absolute lymphocytic count; BMI, body mass index; CI, confidence interval; CLL, chronic lymphocytic leukemia; ECOG PS, eastern cooperative oncology group performance status; eGFR, estimated glomerular filtration rate; HDL-C, high density lipoprotein cholesterol; Hb, hemoglobin; LDL-C, low density lipoprotein cholesterol; OR, odds ratio; PLT, platelet; PS, performance status; TC, total cholesterol; TG, triglyceride.

Supplemental Table 4. Echocardiographic features of the mice administrated with vehicle or ibrutinib for 28 days

	Vehicle (n=8)	Ibrutinib (n=8)	*P* value
E/A	1.55±0.026	1.40±0.044*	0.0119
EF	63.53±0.546	47.18±1.711**	<0.0001
FS (%)	33.68±0.416	23.36±1.037**	<0.0001

E/A, ratio of early left ventricular diastolic flow velocity and late left ventricular diastolic flow velocity; EF, ejection fraction; FS, fractional shortening; **P*<0.05, ***P*<0.01 versus vehicle-treated mice, unpaired *t* test with or without Welch's correction.

Supplemental Table 5. Summary of the parameters of ACh, SNP or nifedipine-induced vasorelaxation in the mesenteric arteries

	ACh			**SNP**			**Nifedipine**		
	$logIC_{50}$	Hill slope	n	$logIC_{50}$	Hill slope	n	$logIC_{50}$	Hill slope	n
MAs from vehicle- or ibrutinib-treated mice									
Vehicle	-5.89±0.18	-0.30±0.04	10	-6.76±0.14	-0.31±0.04	8	-7.86±0.06	-0.52±0.03	11
Ibrutinib	-5.97±0.22	-0.32±0.06	9	-6.50±0.12	-0.32±0.03	8	-8.37±0.09	-0.47±0.04	10
P value	0.7752	0.8482		0.1896	0.8500		<0.0001	0.3462	
Vehicle or ibrutinib-incubated MAs from rats									
Control	-6.12±0.16	-0.34±0.05	11	-6.85±0.10	-0.42±0.046	9	-7.86±0.05	-0.51±0.03	9
Ibrutinib	-6.04±0.11	-0.32±0.03	10	-6.91±0.12	-0.42±0.04	8	-8.19±0.04	-0.56±0.03	9
P value	0.6642	0.6187		0.6966	0.9042		<0.0001	0.2241	

IC_{50}: Half-maximal inhibitory concentration (used here to represent the concentration for 50% vasorelaxation); Hill slope: Slope factor of the concentration-response curve, indicating the steepness of the curve and cooperativity of ligand binding; n: number of arteries per group. *P* values were determined by unpaired *t* test. ACh: acetylcholine; SNP: sodium nitroprusside.

Supplemental Table 6. Summary of Cav1.2 channel electrophysiological kinetics of isolated VSMCs from vehicle- or ibrutinib-treated mice

	$V_{0.5}$ (mV)	E_{rev} (mV)	k	n
Vehicle	-0.89±3.47	52.51±1.36	-16.85±1.19	28
Ibrutinib	-6.67±2.32	51.31±1.12	-15.81±0.88	40
P value	0.1544	0.4970	0.4751	

$V_{0.5}$: half-activation potential, E_{rev}: reversal potential, k: slope rate. Unpaired *t* test.

Supplemental Table 7. Summary of Cav1.2 channel electrophysiological kinetics of vehicle- or ibrutinib-treated VSMCs

	$V_{0.5}$ (mV)	E_{rev} (mV)	k	n
Ctrl	-8.15±3.12	60.02±2.66	-14.81±1.22	30
Ibrutinib	-14.67±2.39	60.23±2.48	-13.54±1.12	30
P value	0.1025	0.9542	0.4463	

$V_{0.5}$: half-activation potential, E_{rev}: reversal potential, k: slope rate. Unpaired *t* test.

Supplemental Table 8. The antihypertensive therapy regimens of the patients with controlled or worsened hypertension when receiving ibrutinib

	Patient No.	Age	Sex	Prior antihypertensive medications	Prior antihypertensive regimens	Hypertension worsening during ibrutinib therapy
CCB-containing regimens	1	60	F	Amlodipine besylate	DHP CCB	Y
	2	64	M	Nifedipine controlled-release tablet	DHP CCB	N
	3	67	M	Amlodipine besylate	DHP CCB	Y
	4	77	M	Amlodipine besylate	DHP CCB	N
	5	83	M	Amlodipine besylate + metoprolol extended-release tablet	DHP CCB + β-blocker	N
	6	68	M	Amlodipine besylate	DHP CCB	N
	7	66	F	Amlodipine besylate	DHP CCB	N
	8	38	M	Nifedipine controlled-release tablet	DHP CCB	N
	9	54	M	Felodipine extended-release tablet	DHP CCB	N
	10	61	F	Nifedipine controlled-release tablet	DHP CCB	N
	11	77	M	Felodipine extended-release tablet	DHP CCB	N
	12	58	M	Amlodipine besylate	DHP CCB	N
	13	85	M	Felodipine + valsartan	DHP CCB + ARB	N
	14	66	M	Amlodipine besylate + losartan potassium/hydrochlorothiazide	DHP CCB + ARB + Diuretic	N
	15	56	F	Nifedipine controlled-release tablet	DHP CCB	N
	16	69	M	Verapamil + losartan + nitrendipine	Non-DHP CCB + DHP CCB + ARB	N
	17	81	F	Amlodipine besylate + metoprolol extended-release tablet	DHP CCB + β-blocker	N
	18	69	M	Amlodipine besylate	DHP CCB	N

CCB-free regimens	1	77	M	Perindopril	ACEI	N
	2	71	M	Valsartan/hydrochlorothiazide	ARB + Thiazide diuretic	Y
	3	68	F	Irbesartan + bisoprolol	ARB + β-blocker	N
	4	65	M	Valsartan	ARB	N
	5	57	F	Metoprolol	β-blocker	N
	6	66	M	Valsartan	ARB	N
	7	63	M	Valsartan	ARB	Y
	8	56	F	Candesartan	ARB	N
	9	54	M	Valsartan	ARB	N
	10	53	F	Irbesartan	ARB	Y
	11	63	M	Irbesartan/hydrochlorothiazide	ARB + Thiazide diuretic	N
	12	80	M	Irbesartan/hydrochlorothiazide	ARB + Thiazide diuretic	Y
	13	48	M	Losartan potassium	ARB	Y
	14	58	M	Telmisartan	ARB	N
	15	60	M	Valsartan	ARB	N
	16	57	M	Losartan potassium	ARB	N
	17	60	F	Valsartan + indapamide	ARB + Thiazide-like diuretic	Y
	18	71	M	Irbesartan	ARB	Y
	19	65	M	Losartan potassium	ARB	Y
	20	70	M	Valsartan	ARB	Y
	21	62	M	Losartan potassium	ARB	Y
	22	51	M	Metoprolol	β-blocker	Y

CCB: calcium channel blocker; DHP: dihydropyridine; non-DHP: non-dihydropyridine; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; F, female; M, male; Y, yes; N, no.

Supplemental Table 9. Summary of Cav1.2 channel electrophysiological kinetics of differentially-treated VSMCs

	$V_{0.5}$ (mV)	E_{rev} (mV)	k	n
Vehicle + NT siRNA	6.28±12.52	55.03±3.87	-18.87±3.53	19
Ibrutinib + NT siRNA	-1.58±4.58	55.11±3.06	-12.49±1.82	21
Vehicle + C/EBP-β siRNA	3.47±7.70	60.33±4.87	-15.25±2.45	19
Ibrutinib + C/EBP-β siRNA	-5.11±3.60	55.57±2.57	-12.85±1.48	20
P value	0.6614	0.6974	0.3737	

$V_{0.5}$: half-activation potential, E_{rev}: reversal potential, k: slope rate. 1-way ANOVA followed by Tukey's multiple comparisons or Brown-Forsythe ANOVA followed by Dunnett's T3 multiple comparisons.

Supplemental Table 10. Major Resources Table

Animals

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse	Animal core facility of NMU	C57BL/6J	Male	-
Rat	Animal core facility of NMU	Sprague Dawley	Male	-

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Persistent ID / URL
anti rabbit-$Ca_V1.2$ α_{1C}	Alomone	#ACC-003	1:1,000	RRID: AB_2039771
anti rabbit-C/EBP-β	Thermo Fisher Scientific	#PA5-27244	1:1,000	RRID: AB_2544720
anti rabbit-C/EBP-β	Proteintech	#23431-1-AP	1:1,000	RRID: AB_2879278
anti mouse-Na-K ATPase	Abcam	#Ab7671	1:1,000	RRID: AB_306023
anti rabbit-β-actin	Proteintech	# 66009-1-IG	1:5,000	RRID: AB_2687938
anti rabbit-CK2	Proteintech	#10992-1-AP	1:1,000	RRID: AB_2245531
anti rabbit-HSP90β	Proteintech	#11405-1-AP	1:1,000	RRID: AB_2121207
anti rabbit-phospho-HSP90β	Sigma-Aldrich	#SAB4300158	1:500	RRID: AB_10621710
anti normal rabbit IgG	Beyotime	#A7016	1:100	RRID: AB_2905533
HRP-conjugated goat anti-mouse IgG	Proteintech	#SA00001-1	1:10,000	RRID: AB_2722565
HRP-conjugated goat anti-rabbit IgG	Proteintech	#SA00001-2	1:10,000	RRID: AB_2722564

DNA/cDNA clones

Name	Vendor or Source	Source / Repository	Persistent ID / URL
pCDNA3.1-*Cebpb*-GFP	Constructed by Genechem Biotechnology Co., Ltd.	GeneChem	-
GV238-*Cacna1c*-promoter	Constructed by Genechem Biotechnology Co., Ltd.	GeneChem	-
GV238-*Cacna1c*-promoter (-1500)	Constructed by Genechem Biotechnology Co., Ltd.	GeneChem	-
GV238-*Cacna1c*-promoter (-1000)	Constructed by Genechem Biotechnology Co., Ltd.	GeneChem	-
pcDNA3.1(+)-*hCSNK2A1*-3×FLAG-T2A-EGFP	Constructed by Umine Biotechnology Co., LTD	Umine	-
pcDNA3.1(+)-*hCSNK2A1*$^{L45A/N118A/I174A}$-3FLAG-T2A-EGFP	Constructed by Umine Biotechnology Co., LTD	Umine	-

Software

Description	Source / Repository	Persistent ID / URL
Graph Pad Prism 10	GraphPad Software	https://www.graphpad-prism.cn/
SPSS Statistics 25	IBM	https://www.ibm.com/products/spss-statistics
pClamp	Molecular Devices	https://www.moleculardevices.com
Clampfit	Molecular Devices	https://www.moleculardevices.com
ImageJ	GitHub	https://imagej.net/ij/download.html
AutoDock Vina1.2.3	Center for Computational Structural Biology	https://autodock.scripps.edu/
PyMOL3.1	PyMOL by Schrödinger	https://pymol.org/
LabChart8	ADINSTRUMENTS	https://www.adinstruments.com/support/software
ZEISS ZEN 3.11	ZEISS	https://www.zeiss.com/microscopy/zh/service-support/downloads.html
Figdraw2.0	Figdraw	http://www.figdraw.com/ ID: OYORR19615

Other (Chemicals)

Description	Source / Repository	Persistent ID / URL
ibrutinib	Selleckchem	#S2680
acalabrutinib	MCE	#HY-17600
acetylcholine;	MCE	#HY-B0282
sodium nitroprusside	MCE	#HY-A0119
nifedipine	MCE	#HY-B0284
SBE-β-CD	MCE	#HY-17031
EGTA	Sigma	#E3889
Mg-ATP	Sigma	#A9187
DMSO	Sigma	#472301
HEPES	Sigma	#H3375
CsCl	Sigma	#C4036
$BaCl_2$	Sigma	#342920
CsOH	Sigma	#232041

Supplemental Table 11. Sequences of siRNAs targeting with C/EBP-β mRNA

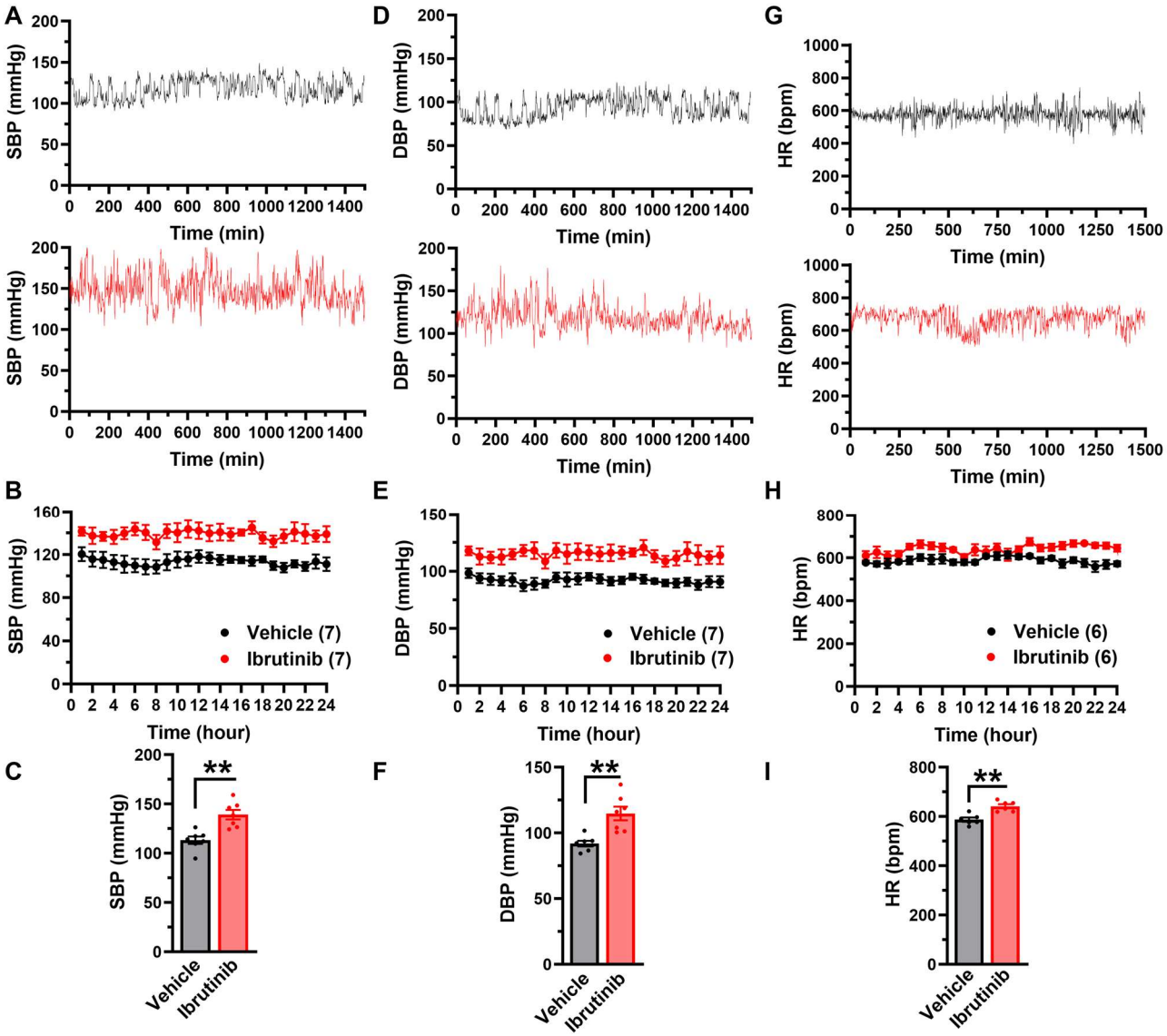
siRNAs	Sense/antisense	Sequences
#1 siRNA	Sense	5′ CGCCUUUAGACCCAUGGAAdTdT 3′
	Antisense	3′ dTdTGCGGAAAUCUGGGUACCUU 5′
#2 siRNA	Sense	5′ GGAACUUGUUCAAGCAGCUdTdT 3′
	Antisense	3′ dTdTCCUUGAACAAGUUCGUCGA 5′
#3 siRNA	Sense	5′ ACCUCUUCGCCGACGACUAdTdT 3′
	Antisense	3′ dTdTUGGAGAAGCGGCUGCUGAU 5′

Supplemental Table 12. Primer sequences for PCR

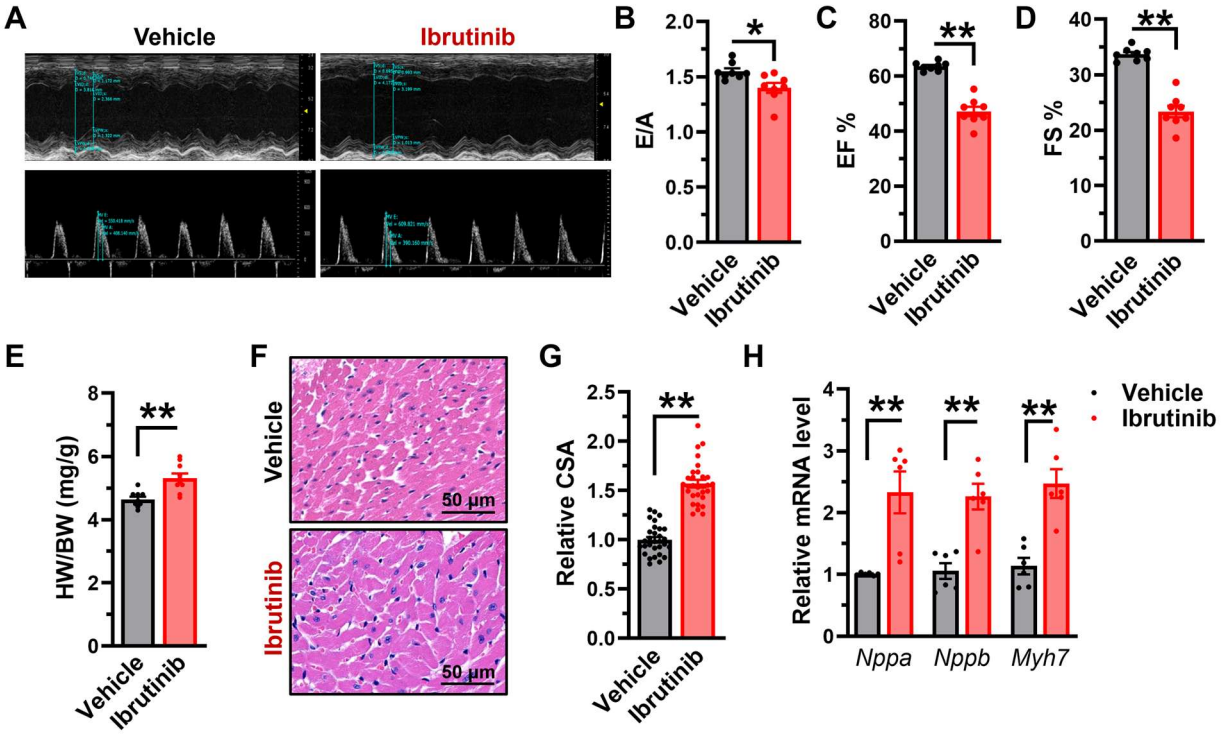
Target genes	Oligonucleotide primers (5'-3')	Product length (bp)
Rat *Cacna1c**	FP: GAGAGCTTTCCGTGTGCTTC RP: GTTCAGGACCACCTGGAGAC	70
Mouse *Cacna1c**	FP: GAGAGCGTTCCGCGTGCTCC RP: GTTCAGGACCACCTGGAGAC	70
Rat *Cacna1c*#*	FP: AATGTTGGAAGGTTAGGTGTGAC RP: AGACAGCTTACTTGGAAAATGGT	100
Rat *Cebpb**	FP: AAGGCGGTGGACAAGCTGAG RP: GGCAGCTGCTTGAACAAGTTC	212
Mouse *Cebpb**	FP: AAGACGGTGGACAAGCTGAG RP: GGCAGCTGCTTGAACAAGTTC	212
Rat *Gapdh**	FP: CAGGGCTGCCTTCTCTTGTG RP: AACTTGCCGTGGGTAGAGTC	111
Mouse *Gapdh**	FP: CAGGGCTGCCATTTGCAGTG RP: AATTTGCCGTGAGTGGAGTC	111
Mouse *Nppa**	FP: AGCTTGGTCACATTGCCACT RP: AGAGGCAAGACCCCACTAGA	119
Mouse *Nppb**	FP: GAAGGACCAAGGCCTCACAA RP: ACTTCAGTGCGTTACAGCCC	176
Mouse *Myh7**	FP: CTACCCTCAGGTAGGAGTGGG RP: TGGTACTACATGCCAGAAGCC	109

*These primers are used for real-time PCR;

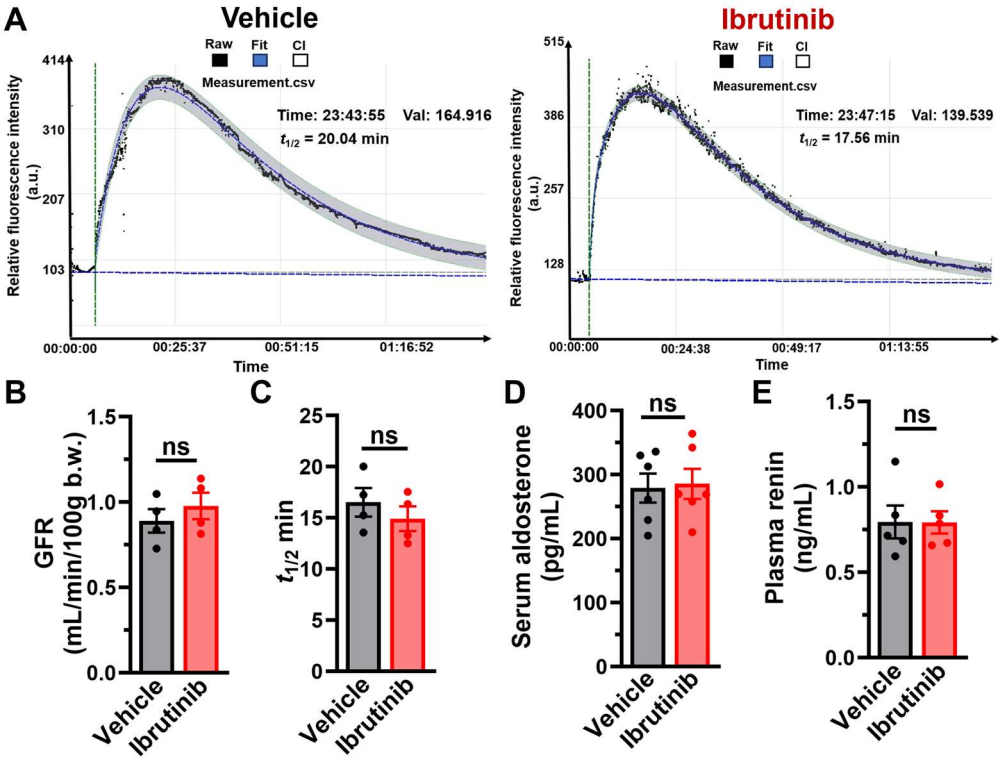
#These primers are used for real-time PCR following CUT & RUN assay.



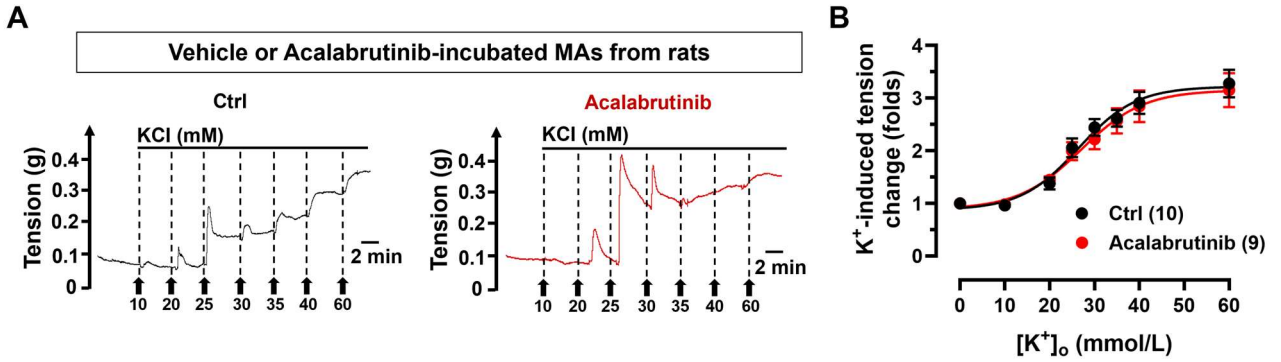
Supplemental Figure 1. Arterial blood pressure is measured for 24 hours in the conscious mice receiving 28 day-treatment with vehicle or ibrutinib by using radiotelemetry. Representative traces (**A, D** and **G**) and corresponding summary data (**B, E** and **H**) for 24-hr recordings (12:00 pm to 12:00 pm the next day) of systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) (1-min intervals) in freely moving conscious mice treated with vehicle (n=7 or 6) or ibrutinib (n=7 or 6). SBP (**C**), DBP (**F**) and HR (**I**) in vehicle and ibrutinib-treated mice was analyzed and presented as a bar chart. ***P*<0.01, unpaired t test with or without Welch's correction.



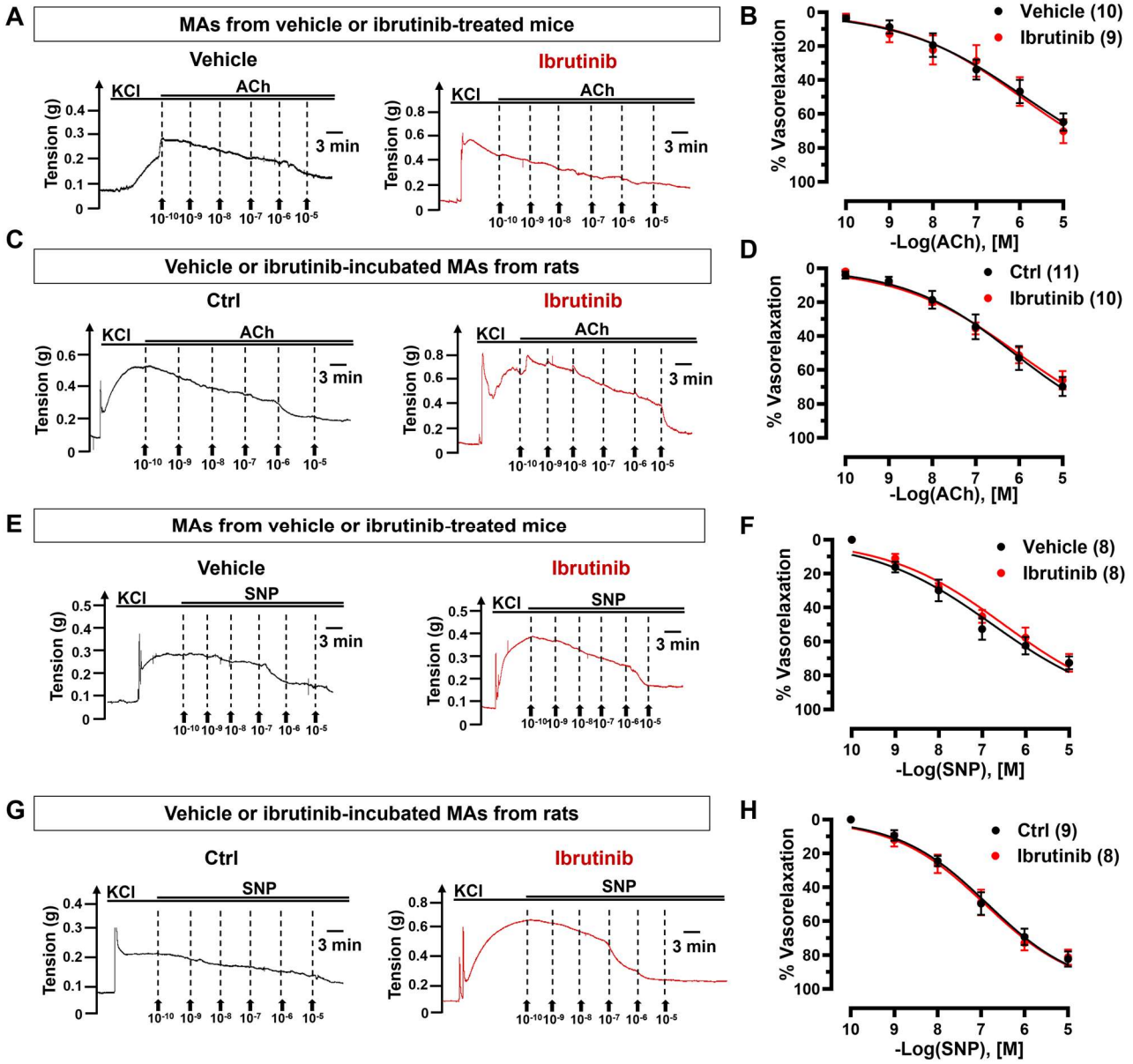
Supplemental Figure 2. Ibrutinib decreases cardiac functions and induces cardiac hypertrophy in mice. (**A**) Representative M-mode traces and left ventricle (LV) Doppler images in vehicle or ibrutinib-treated mice. (**B**) E/A ratios (ratio of early left ventricular diastolic flow velocity and late left ventricular diastolic flow velocity) were analyzed and presented as a bar chart. n=8 mice for each group. **P*=0.0119, unpaired *t* test. Ejection fraction (EF) (**C**) and fractional shortening (FS) (**D**) were also analyzed and presented as bar charts. n=8 mice for each group. ***P*<0.01, unpaired *t* test with Welch's correction. (**E**) Heart weight-to-body weight (HW/BW) ratios in vehicle- (n=9) or ibrutinib-treated mice (n=10) were analyzed and presented as a bar chart. ***P*=0.0008, unpaired *t* test. (**F**) Representative images of H&E staining for cardiac tissue from the mice after ibrutinib or vehicle treatment. (**G**) Cross-sectional area (CSA) was analyzed and showed as a bar chart. n=30 cells from 3 mice for each group. ***P*<0.0001, unpaired *t* test. (**H**) Mouse *Nppa*, *Nppb* and *Myh7* mRNA levels were determined by real-time RT-qPCR, and mouse *Actb* mRNA was measured as internal control. n=6 mice for each group. ***P*<0.01, unpaired *t* test.



Supplemental Figure 3. Ibrutinib treatment does not impair glomerular filtration or activate the renin-angiotensin-aldosterone system. (**A**) Representative real-time transcutaneous fluorescence intensity curves of the fluorescent tracer in vehicle- or ibrutinib-treated mice. The elimination kinetics of the tracer (measured as relative fluorescence intensity) over time reflects the glomerular filtration rate (GFR). The GFR (**B**) and clearance half-life ($t_{1/2}$) (**C**) were analyzed and presented as a bar chart derived from the clearance curves in vehicle- and ibrutinib-treated mice. n=4 mice for each group. Serum aldosterone (**D**) and plasma renin (**E**) levels in mice treated with vehicle or ibrutinib were analyzed by ELISA and presented as a bar chart. n=6 or 5 mice per group. ns indicates no significant differences, unpaired *t* test.

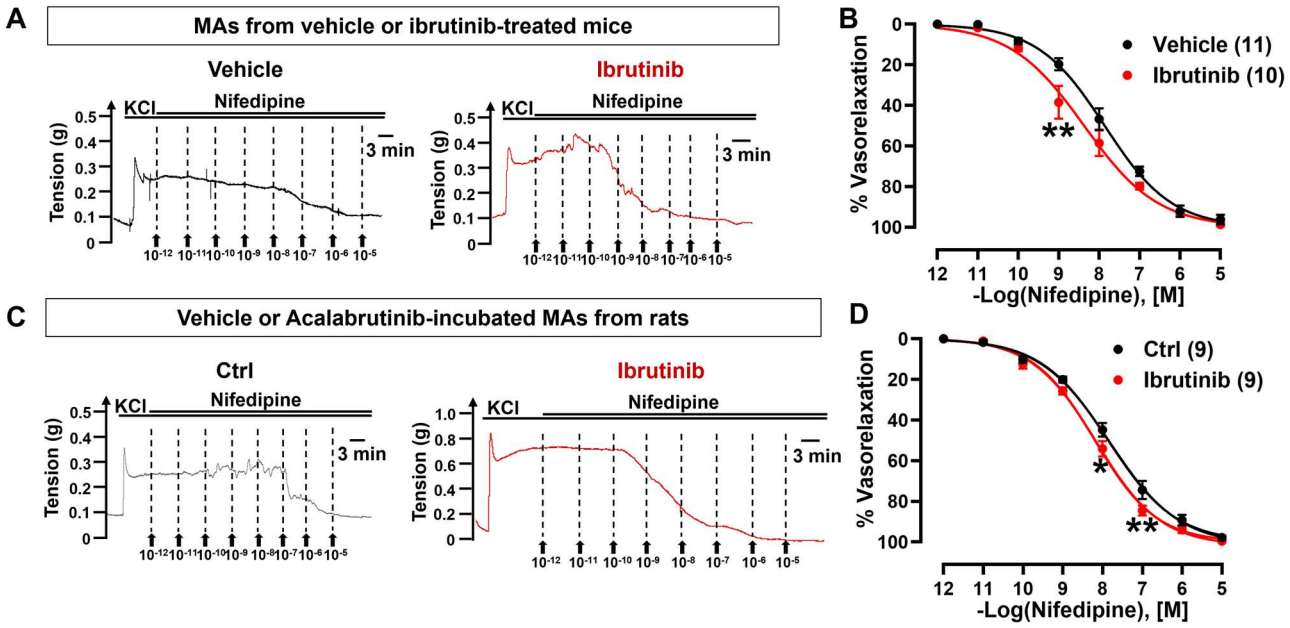


Supplemental Figure 4. The second-generation BTK inhibitor acalabrutinib does not alter K^+-induced vasoconstriction in MAs. (**A**) Representative traces of mesenteric artery tension responding to increased KCl extracellular concentration (from 0 to 60 mmol/L KCl) by using vascular myograph in MAs from rats after 48-hr incubation with vehicle or acalabrutinib (1 μmol/L). (**B**) Plots of concentration-tension relationship were represented for vehicle- (n=10 arteries from 4 rats) or acalabrutinib-incubated (n=9 arteries from 4 rats) MAs. There are no significant differences, 2-way ANOVA followed by Sidak's multiple comparisons.

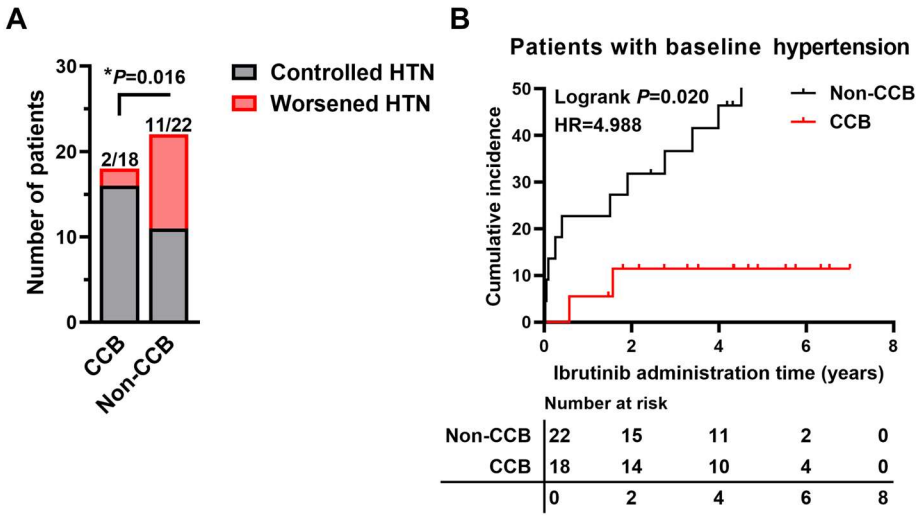


Supplemental Figure 5. Ibrutinib does not affect endothelium- or nitric oxide (NO)-dependent vasorelaxation. (**A**) Representative traces of mesenteric artery (MA) tension responding to different concentration of acetylcholine (ACh) (10^{-10} to 10^{-5} mol/L) under 30 mmol/L KCl bath solution using vascular myography in vehicle- or ibrutinib-treated mice. (**B**) Plots of concentration-tension relationship were represented for the MAs from vehicle (n=10 arteries from 4 mice) or ibrutinib-treated (n=9 arteries from 4 mice) mice. (**C**) Representative traces of ACh-induced vasodilatation of isolated MAs after 48-hr incubation with vehicle or ibrutinib (1 μmol/L). (**D**) Concentration-response curves were presented for control (n=11 arteries from 4 rats) or ibrutinib-incubated (n=10 arteries from 4 rats) MAs. (**E**) Traces of MA tension in response to different concentration of sodium nitroprusside (SNP) (10^{-10} to 10^{-5} mol/L) under 30 mmol/L KCl bath solution using vascular myography in vehicle- or ibrutinib-treated mice are shown. (**F**) Plots of concentration-tension relationship were represented for

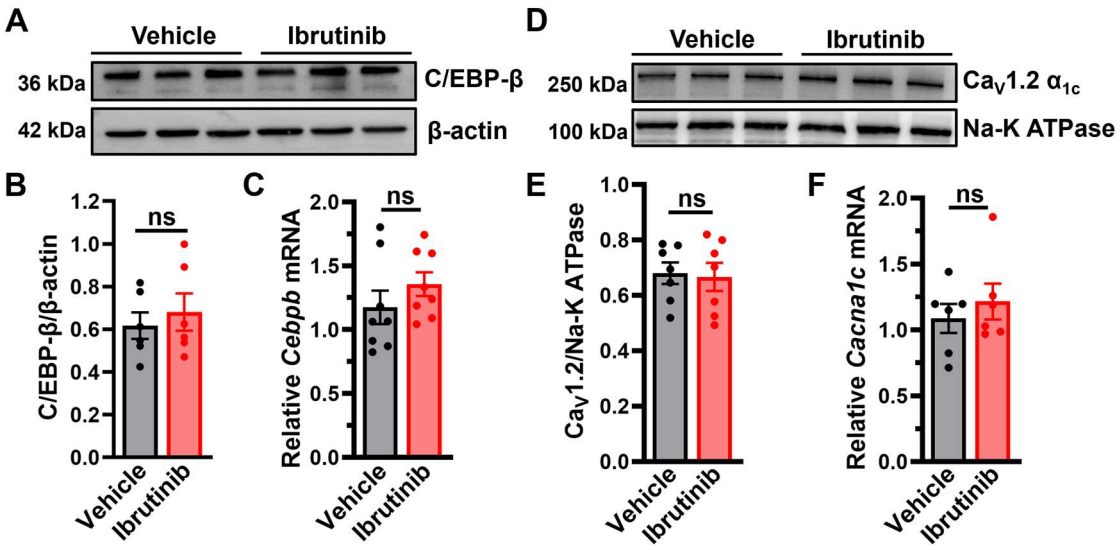
the MAs from vehicle (n=8 arteries from 3 mice) or ibrutinib-treated (n=8 arteries from 3 mice) mice. (**G**) Representative traces of SNP-induced vasodilatation of isolated MAs after 48-hr incubation with vehicle or ibrutinib (1 μmol/L). (**H**) Concentration-response curves were presented for control (n=9 arteries from 4 rats) or ibrutinib-incubated (n=8 arteries from 4 rats) MAs. There are no significant differences, 2-way ANOVA followed by Sidak's multiple comparisons.



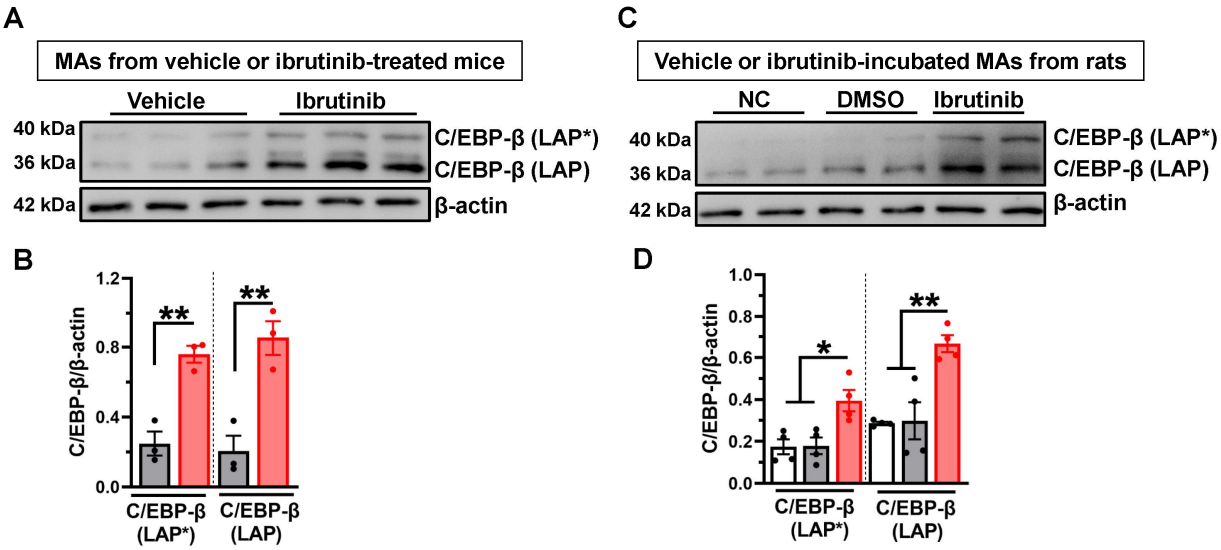
Supplemental Figure 6. Ibrutinib treatment increases the sensitivity of nifedipine-induced vasorelaxation in mesenteric arteries. (**A**) Arterial tension responding to different concentration of nifedipine (10^{-12} to 10^{-5} mol/L) under 30 mmol/L KCl bath solution was measured by vascular myography in vehicle or ibrutinib-treated mice. (**B**) Plots of concentration-tension relationship were represented for the MAs from mice treated with vehicle (n=11 arteries from 4 mice) or ibrutinib (n=10 arteries from 4 mice). (**C**) Representative traces of nifedipine-induced vasodilatation of isolated MAs after 48-hr incubation with vehicle or ibrutinib (1 μmol/L). (**D**) Concentration-response curves were presented for vehicle (n=9 arteries from 3 rats) or ibrutinib-incubated (n=9 arteries from 3 rats) MAs. **P*<0.05, ***P*<0.01, 2-way ANOVA with Sidak's test.



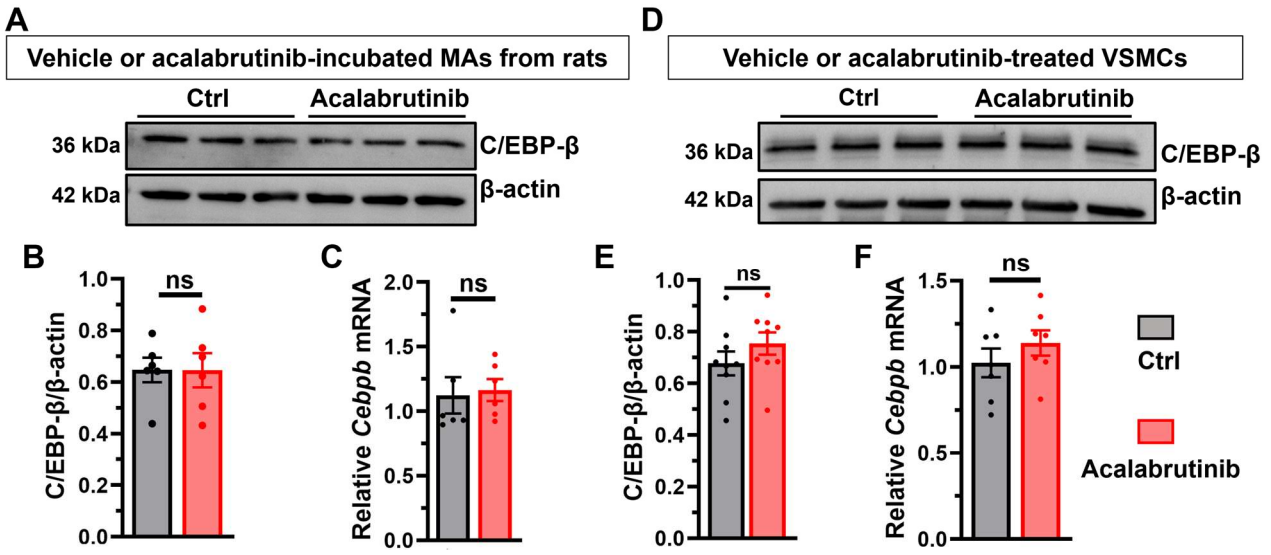
Supplemental Figure 7. Therapy with calcium channel blockers reduces the incidence of hypertension in CLL patients receiving ibrutinib. (**A**) Number of patients with controlled or worsened hypertension receiving the antihypertensive therapy regimen including calcium channel blocker (CCB) or non-CCB, **P*=0.016, chi-square test. (**B**) Cumulative incidence of worsened hypertension treated with antihypertensive therapy regimens including CCBs or non-CCB drugs in ibrutinib-treated patients with baseline hypertension during the follow-up period. HR, hazard ratio, log-rank test.



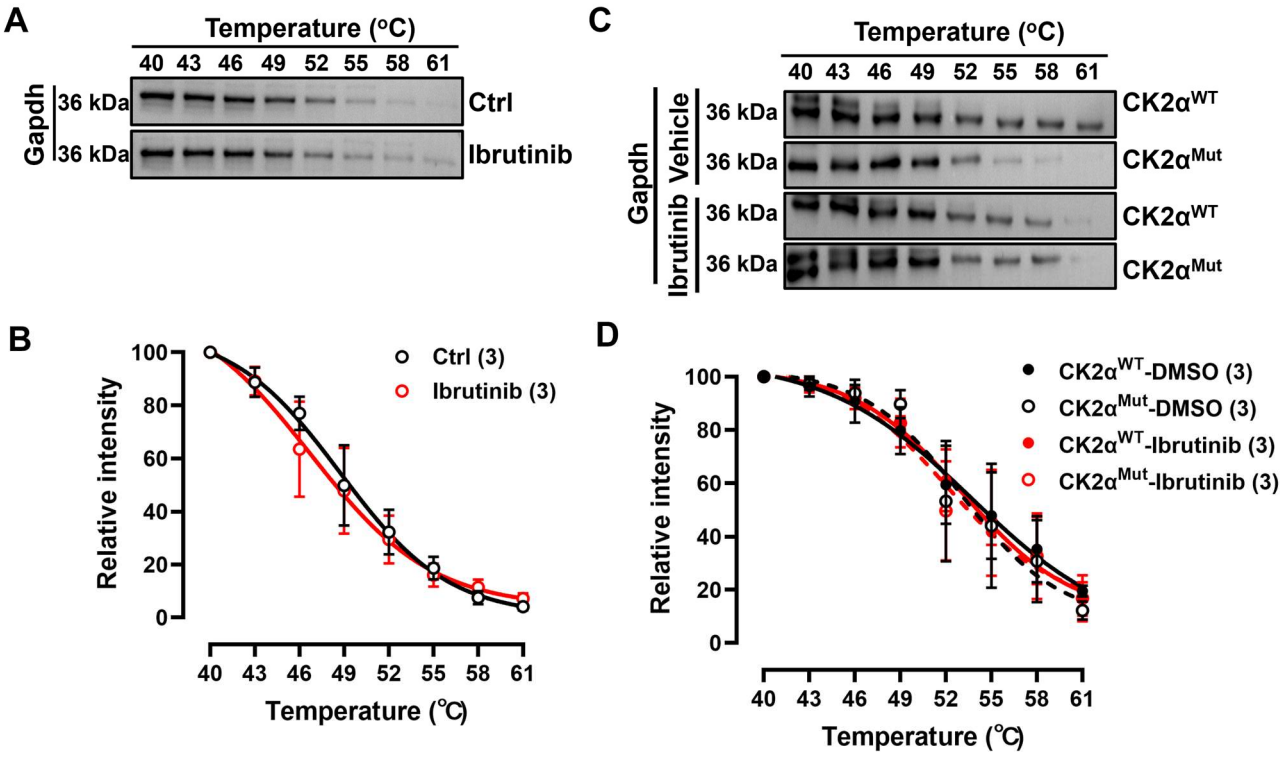
Supplemental Figure 8. Ibrutinib does not alter C/EBP-β or Cav1.2 channel expression in the heart. (**A**) Representative immunoblots of C/EBP-β protein in heart tissues from the mice treated with or without ibrutinib, β-actin was detected as loading control. (**B**) Quantified C/EBP-β expression was analyzed and presented as a bar chart. n=6 mice per group. (**C**) *Cebpb* mRNA levels in heart tissues were analyzed. n=8 mice per group. (**D**) The membrane expression of $Ca_V1.2$ α_{1C} protein was detected by Western blotting in heart tissues from vehicle- and ibrutinib-treated mice, Na-K ATPase protein was detected as internal control. (**E**) Quantified $Ca_V1.2$ α_{1C} protein expression was analyzed and presented as a bar chart. n=7 mice per group. (**F**) *Cacna1c* mRNA levels in heart tissues from the differentially-treated mice were presented as a bar chart. n=6 mice per group. ns indicates no significant differences, unpaired *t* test.



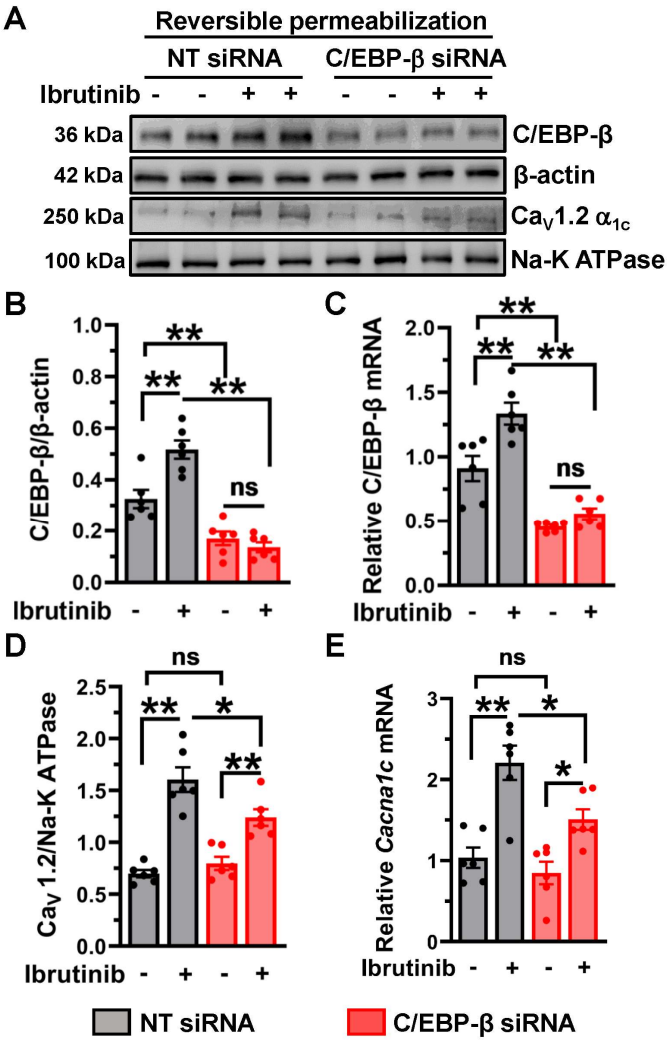
Supplemental Figure 9. Ibrutinib increases the expression of C/EBP-β isoforms. (**A**) Expressions of C/EBP-β isoforms in mesenteric arteries (MAs) from the mice administrated with or without ibrutinib. (**B**) Quantified activating protein* (LAP*) and LAP isoforms of C/EBP-β in MAs were presented. n=3 independent experiments per group. (**C**) Expressions of C/EBP-β isoforms in isolated VSMCs post 48-hr treatment with or without 1 μmol/L ibrutinib. (**D**) Quantified activating protein* (LAP*) and LAP isoforms of C/EBP-β were analyzed and presented. n=4 independent experiments per group. **P*<0.05, ***P*<0.01, 1-way ANOVA with Tukey's test.



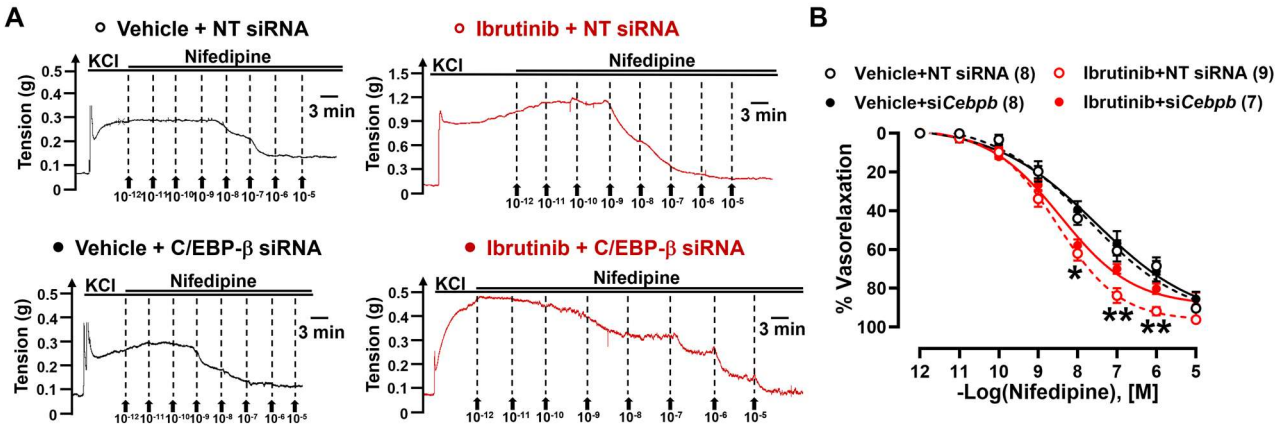
Supplemental Figure 10. The second-generation BTK inhibitor, acalabrutinib, does not induce C/EBP-β expression in MAs and vascular smooth muscle cells. Immunoblots of C/EBP-β in vehicle or acalabrutinib-incubated MAs from rats (**A**) and vehicle or acalabrutinib-treated VSMCs from rats (**D**). β-actin was detected as loading control. Quantified C/EBP-β expression (**B** and **E**) were analyzed and presented as a bar chart. n=6 or 9 independent experiments per group. *Cebpb* mRNA levels were determined by real-time RT-qPCR in MAs (**C**) and isolated VSMCs (**F**) from rats after 48-hr incubation with vehicle or acalabrutinib (1 μmol/L). n=6 or 7 independent experiments per group. ns indicates no significant differences, unpaired *t* test.



Supplemental Figure 11. Ibrutinib does not cause a thermal stability shift in Gapdh. (**A**) Representative immunoblots of Gapdh in cell lysates from VSMCs incubated with DMSO (Ctrl) or 1 μmol/L ibrutinib for 2 hr subjected to a temperature gradient (40–61°C) using the cellular thermal shift assay (CETSA). (**B**) Quantification of the thermal melting curves for Gapdh in ctrl or ibrutinib groups. The relative intensity of the protein bands was plotted against temperature. (**C**) Representative immunoblots of Gapdh in cell lysates from HEK293 cells expressed with wild-type CK2α ($CK2\alpha^{WT}$) or the $CK2\alpha^{L45A/N118A/I174A}$ triple mutant ($CK2\alpha^{Mut}$) incubated with DMSO or ibrutinib by CETSA. (**D**) Thermal stability curve was analyzed and fitted with Boltzmann equation. These are no significant differences, 3-way ANOVA with Tukey's test.



Supplemental Figure 12. C/EBP-β knockdown decreases Cav1.2 channel expression in mesenteric arteries. (**A**) siRNAs were introduced into mesenteric arteries (MAs) by reversible permeabilization protocol, then the MAs were cultured for 72 hr. After that, the expression of C/EBP-β in whole lysate and membrane expression of $Ca_V1.2$ α_{1C} were checked by Western blotting, β actin and Na-K ATPase were detected as loading control, respectively. (**B**) Quantified C/EBP-β protein was analyzed and presented as a bar chart. (**C**) *Cebpb* mRNA levels were checked by real-time RT-PCR. (**D**) Quantified $Ca_V1.2$ α_{1C} protein were analyzed and presented. (**E**) *Cacna1c* mRNA levels were checked in differentially-treated MAs and presented as a bar chart. n=6 independent experiments per group. **P*<0.05, ***P*<0.01, 1-way ANOVA with Tukey's test; ns: not significant.



Supplemental Figure 13. C/EBP-β knockdown abolishes the increased sensitivity of nifedipine-induced vasorelaxation by ibrutinib. (**A**) Representative traces of vasodilation induced by different concentrations of nifedipine (10^{-12} to 10^{-5} mol/L) under 30 mmol/L KCl bath solution were presented in mesenteric arteries (MAs) from mice after 72-hr siRNA introduction upon application with or without ibrutinib. (**B**) Concentration-response curves of nifedipine-induced vasodilatation were presented. n=7-9 arteries from ≥3 mice, **P*<0.05, ***P*<0.01 versus vehicle + NT siRNA, 3-way ANOVA with Tukey's test.