Supplemental Data

Vitamin D Controls Resistance Artery Function through Regulation of Perivascular Adipose Tissue Hypoxia and Inflammation

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Supplemental Figure and Table Legends.

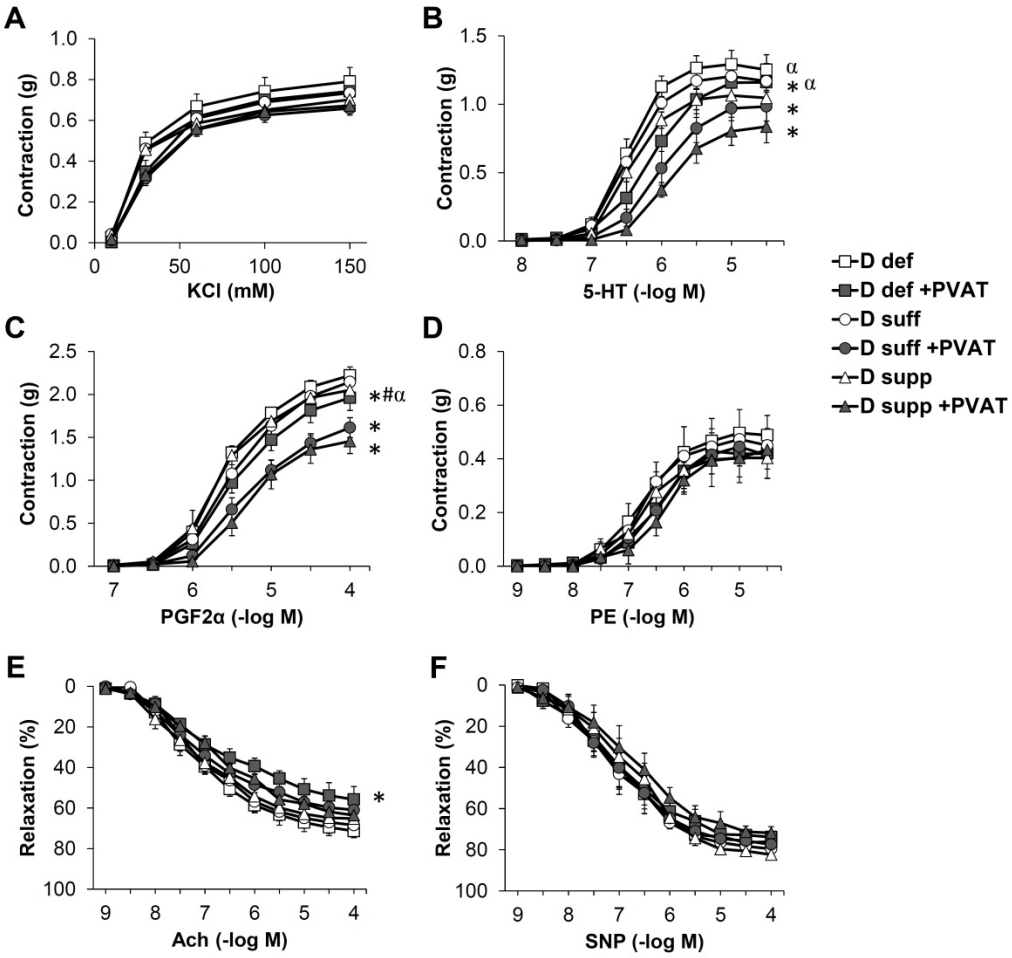

Figure S1: Vitamin D deficiency counteracts attenuates the normal anti-contractile effects PVAT in aortic rings. Isometric tension was measured on aortic rings from mice maintained on vitamin D-deficient diet (D def; squares), vitamin D-sufficient diet (D suff; circles) or vitamin D-supplemented diet (D supp; triangles). The aortic rings were either dissected free of PVAT (open symbols) or had the PVAT remaining intact (filled symbols). Dose-dependent contraction

was assessed in response to KCl (A), serotonin (5-HT) (B), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (C) or phenylephrine (PE) (D). Dose-dependent relaxation was assessed in response to acetylcholine (Ach) (E) and sodium nitroprusside (SNP) (F) following precontraction with $PGF_{2\alpha}$. Data represent mean ± SE (n=5-8). *p<0.05 PVAT vs. no PVAT. $^{\#}$p<0.05 D def or D supp vs. D suff. $^{\alpha}$p<0.05 D def vs. D supp.

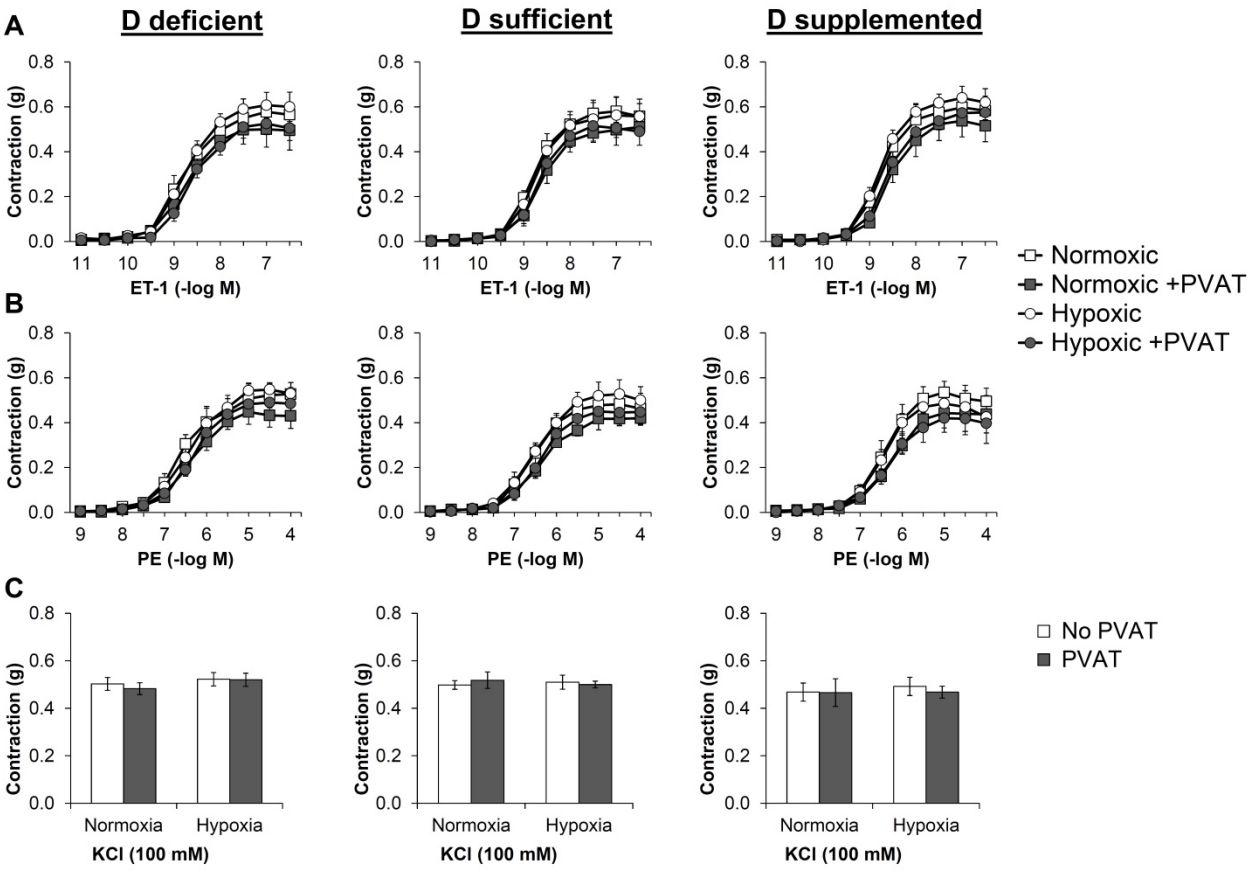

Figure S2: Effects of PVAT and hypoxic incubation on mesenteric artery responses to ET-1, PE and KCl. Isometric tension was measured on first order mesenteric arteries from mice maintained on the three vitamin D diets. The mesenteric artery rings were either dissected free of PVAT (open symbols) or had the PVAT remaining intact (filled symbols) and incubated for 24 hours under normoxic (squares) or hypoxic conditions (circles). Dose-dependent contraction was assessed in response to endothelin 1 (ET-1) (A), PE (B) or KCl (C). Data represent mean ± SE (n=4-5). *p<0.05 PVAT vs. no PVAT. $^{\#}$p<0.05 D def or D supp vs. D suff.

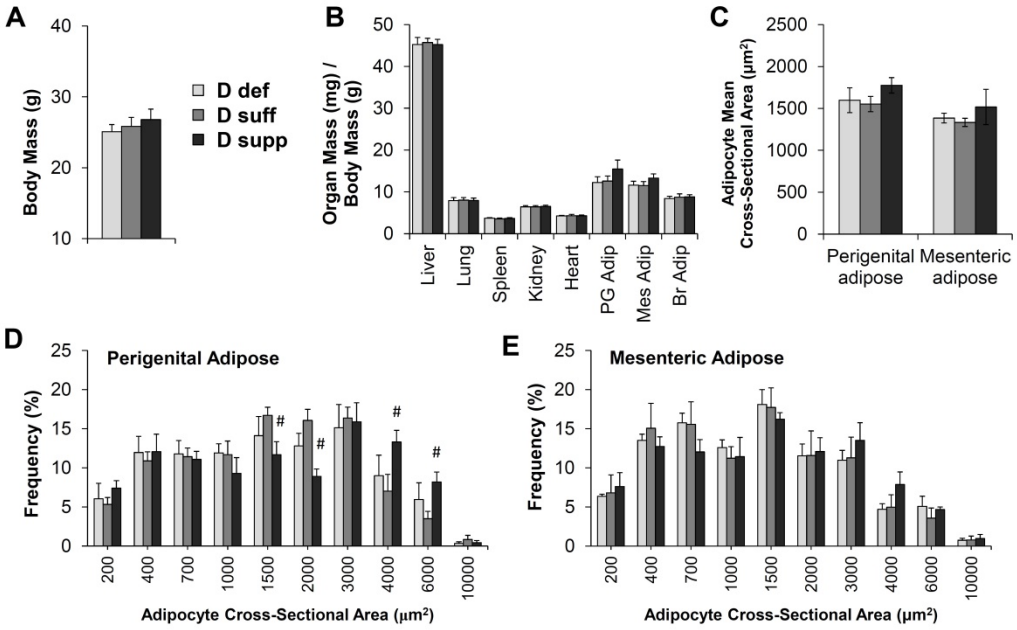

Figure S3: Vitamin D supplementation increases adipocyte size. Organ mass was recorded and normalized to body mass (A-B) (n=14 mice). Adipocyte cross-sectional area (μm^2) was calculated for perigenital and mesenteric adipose tissues (C) (n=5 mice). Distribution histograms were generated for adipocyte cross-sectional area (D-E). Data represent mean ± SE. $^{\#}p<0.05$ D def or D supp vs. D suff.

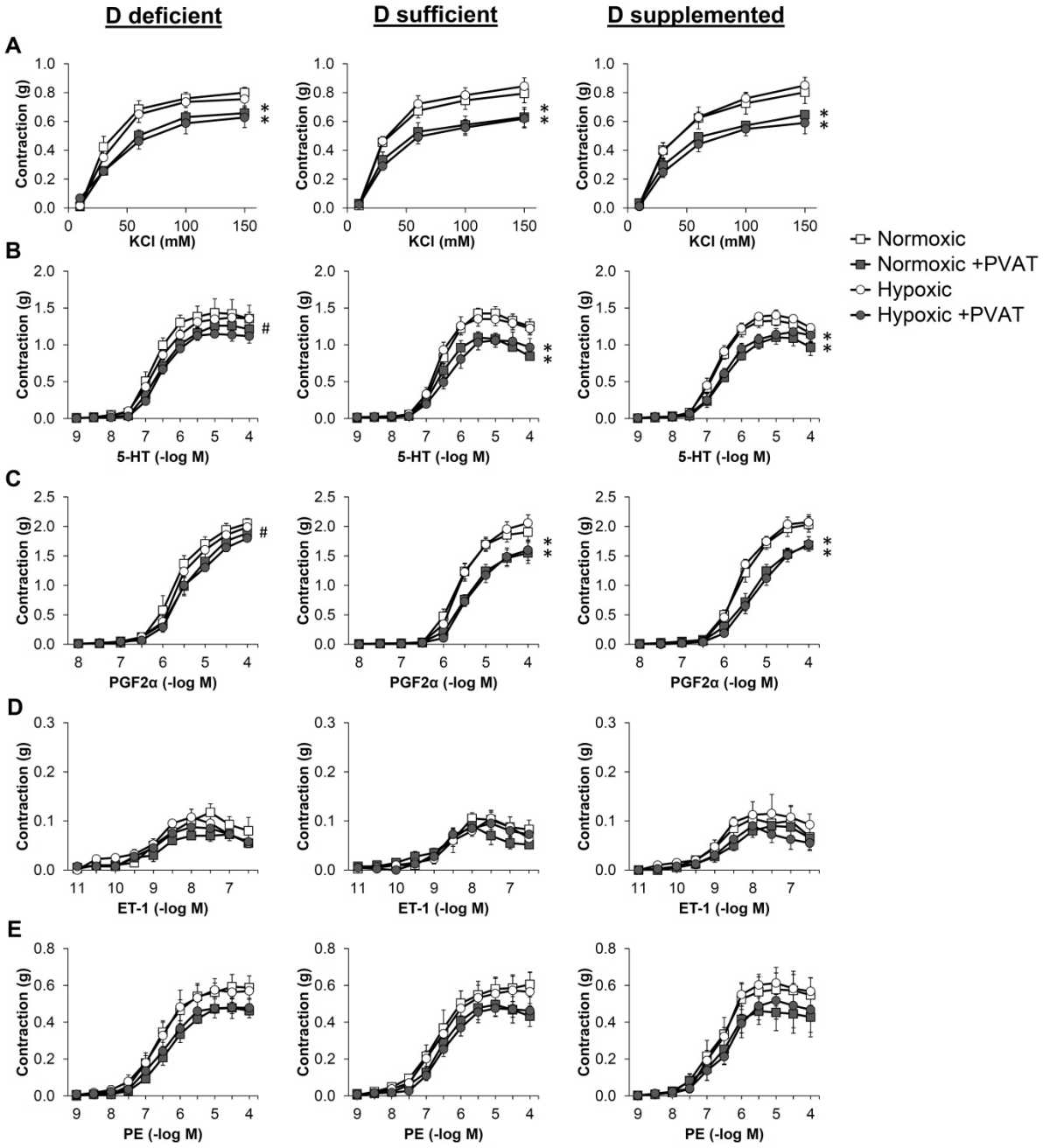

Figure S4: Effects of PVAT and normoxic or hypoxic incubation on aortic contractility. Isometric tension was measured on aortic rings from mice maintained on the three vitamin D diets. The aortic rings were either dissected free of PVAT (open symbols) or had the PVAT remaining intact (filled symbols) and incubated for 24 hours under normoxic (squares) or hypoxic conditions (circles). Dose-dependent contraction was assessed in response to KCl (A), 5-HT (B), $PGF_{2\alpha}$ (C), ET-1 (D) or PE (E). Data represent mean ± SE (n=4-5). *p<0.05 PVAT vs. no PVAT. $^{\#}$p<0.05 D def or D supp vs. D suff.

Table S1: Mass of artery segments from myograph experiments.
Following the myograph experiments, the artery rings were unmounted and blotted dry. The mass (in mg) was recorded for aortic rings with or without intact PVAT and mesenteric artery rings with intact PVAT. The mass of mesenteric artery rings without PVAT was too small to be determined. Data represent mean ± SE (n=4-6). The average mass for each type of artery ring was similar, and there were no significant differences when comparing the three different vitamin D diet groups.

Artery	Thor. Ao.	Thor. Ao.	Mes. 2nd	Thor. Ao.	Thor. Ao.	Mes 1st	Thor. Ao.	Thor. Ao.	Mes 1st
Incubation	None	None	None	Normoxia	Normoxia	Normoxia	Hypoxia	Hypoxia	Hypoxia
PVAT	(-)	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(+)
D def	0.46±0.07	2.72±0.31	0.63±0.04	0.45±0.07	2.73±0.22	0.65±0.06	0.48±0.09	2.55±0.29	0.63±0.09
D suff	0.43±0.04	2.67±0.27	0.63±0.06	0.48±0.06	2.60±0.24	0.58±0.05	0.46±0.05	2.44±0.19	0.62±0.04
D supp	0.44±0.05	2.76±0.30	0.60±0.07	0.46±0.05	2.50±0.25	0.66±0.08	0.50±0.07	2.58±0.22	0.60±0.05