Supplemental figures

Delineating the molecular and histological events that govern right ventricular recovery using a novel mouse model of PA de-banding

Short title: A novel de-PAB model to study RV recovery

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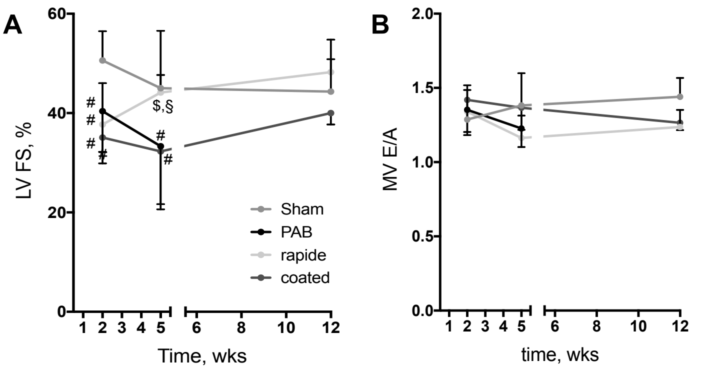
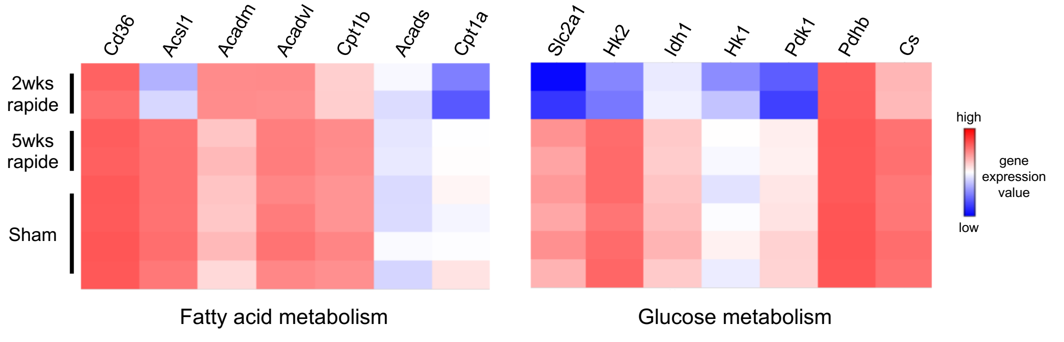


Supplementary Figure 1: Impaired LV function after PAB improves upon de-PAB

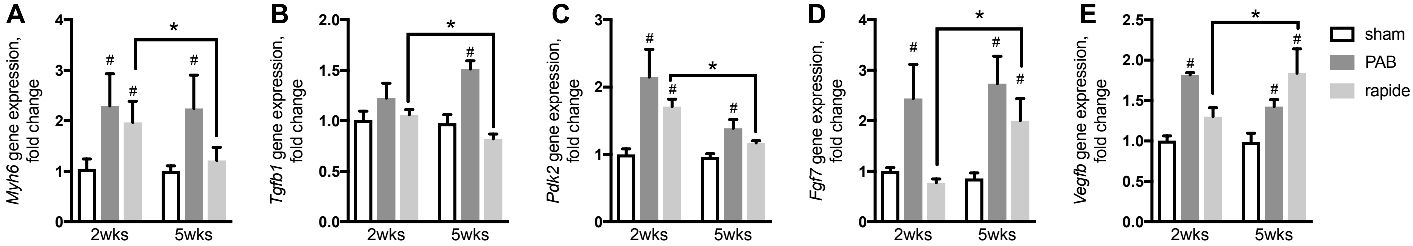
Echocardiographic assessment of LV function (A LV FS: left ventricular fractional shortening; B, MV E/A: ratio of mitral valve peak velocity flow in early diastole (E) to late diastole (A)) longitudinally over 12 weeks revealed that RV recovery from pressure overload is accompanied by normalization in global LV function once the increased afterload burden is relieved. Two-way ANOVA followed by Tukey's multiple comparison post-hoc test was performed; #: $p<0.05$ vs. sham; $: $p<0.05$ rapide vs. PAB; §: $p<0.05$ rapide vs. coated. n=4-11 animals per group and timepoint.



Supplementary Figure 2: Normalization in RV energy metabolism following de-PAB

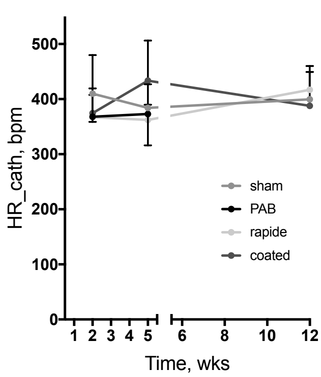
Gene array analysis of RV tissue homogenates revealed a de-regulation in genes encoding key enzymes involved in fatty acid and glucose metabolism 2wks after PA banding with fast absorbing sutures (rapide). Those expression changes returned to levels comparable with sham-operated controls after de-PAB (5wks rapide), suggesting normalization in RV energy metabolism. Especially, expression of genes involved in the fatty acid transport into the cell (Cd36, Acsl1), into mitochondria (Cpt1a, Cpt1b) and fatty acid β-oxidation (Acads, Acadm, Acadvl) normalized together with glycolysis- (Slc2a1, Hk1, Hk2, Pdk1) and aerobic glucose oxidation-associated genes (Idh1, Cs, Pdhb).

Cd36 = cluster of differentiation 36; Acsl1 = Acyl-CoA Synthetase Long Chain Family Member 1; Acadm = Acyl-CoA Dehydrogenase Medium Chain; Acadvl = Acyl-CoA Dehydrogenase Very Long Chain; Cpt1b = Carnitine Palmitoyltransferase 1b; Acads = Acyl-CoA Dehydrogenase Short; Cpt1a = Carnitine Palmitoyltransferase 1a; Slc2a1 = Solute Carrier Family 2 Member 1; Hk2 = Hexokinase 2; Idh1 = Isocitrate Dehydrogenase (NADP(+)); Hk1 = Hexokinase 1; Pdk1 = Pyruvate Dehydrogenase; Pdhb = Pyruvate Dehydrogenase E1 Component Subunit Beta; Cs = Citrate Synthase.



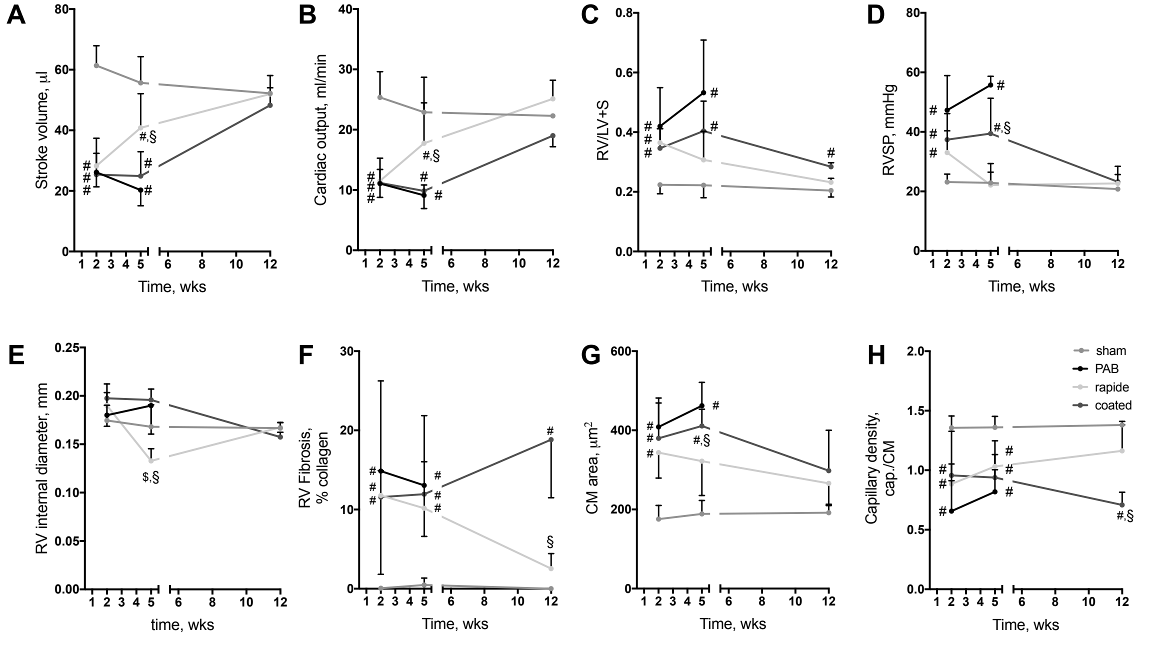
Supplementary Figure 3: Gene array validation of de-regulated genes via qPCR

Myh6, *Tgfb1* and *Pdk2* gene expression are closely linked to hypertrophic (A, *Myh6*), fibrotic (B, *Tgfb1*) and metabolic (C, *Pdk2*) remodeling processes. Here, we demonstrate via qPCR an increase in gene expression for those 3 target genes in response to permanent PAB as compared to sham controls, a response that is blunted once the increased afterload burden is relieved and RV function improves after de-PAB (rapide). Further, we validate the de-regulation of *Fgf7* (D) and *Vegfb* (E), genes whose de-regulation was identified by gene array analysis, in the recovery process, validating the rigidity of the gene array results and suggesting that *Fgf7* and *Vegfb* may play a role in RV recovery processes. Two-way ANOVA followed by Tukey's multiple comparison post-hoc test was performed; #: $p<0.05$ vs. sham; *: $p<0.05$ 2wks vs. 5wks. n=3-4 animals per group and timepoint.



Supplementary Figure 4: Heart rate assessment at the time of intra-cardiac catheterization

Heart rate at the time of catheterization was derived from intra-cardiac RV pressure tracings to demonstrate the overall condition of mice and proper animal handling. Two-way ANOVA was performed without significant differences among all groups and timepoints.



Supplementary Figure 5: Change of key RV structural and functional parameters over time

Assessment of stroke volume (A) and cardiac output (B) reveals normalization of RV function upon de-banding. RV hypertrophy assessed by RV/(LV+S) ratio demonstrates normalization of RV hypertrophy (C) along with RV systolic pressure (RVSP, D) and RV internal diameter (RVID, E) over time. RV fibrosis reversed following de-PAB in rapide but not in coated banded animals at 12 weeks post-surgery (F) while cardiomyocyte hypertrophy (G) but not capillary density (H) normalized in all animals. Two-way ANOVA followed by Tukey's multiple comparison post-hoc test was performed; #: $p<0.05$ vs. sham; §: $p<0.05$ rapide vs. coated. n=4-15 animals per group and timepoint.