Necrosis-released HMGB1 in the progressive pulmonary arterial hypertension associated with male sex

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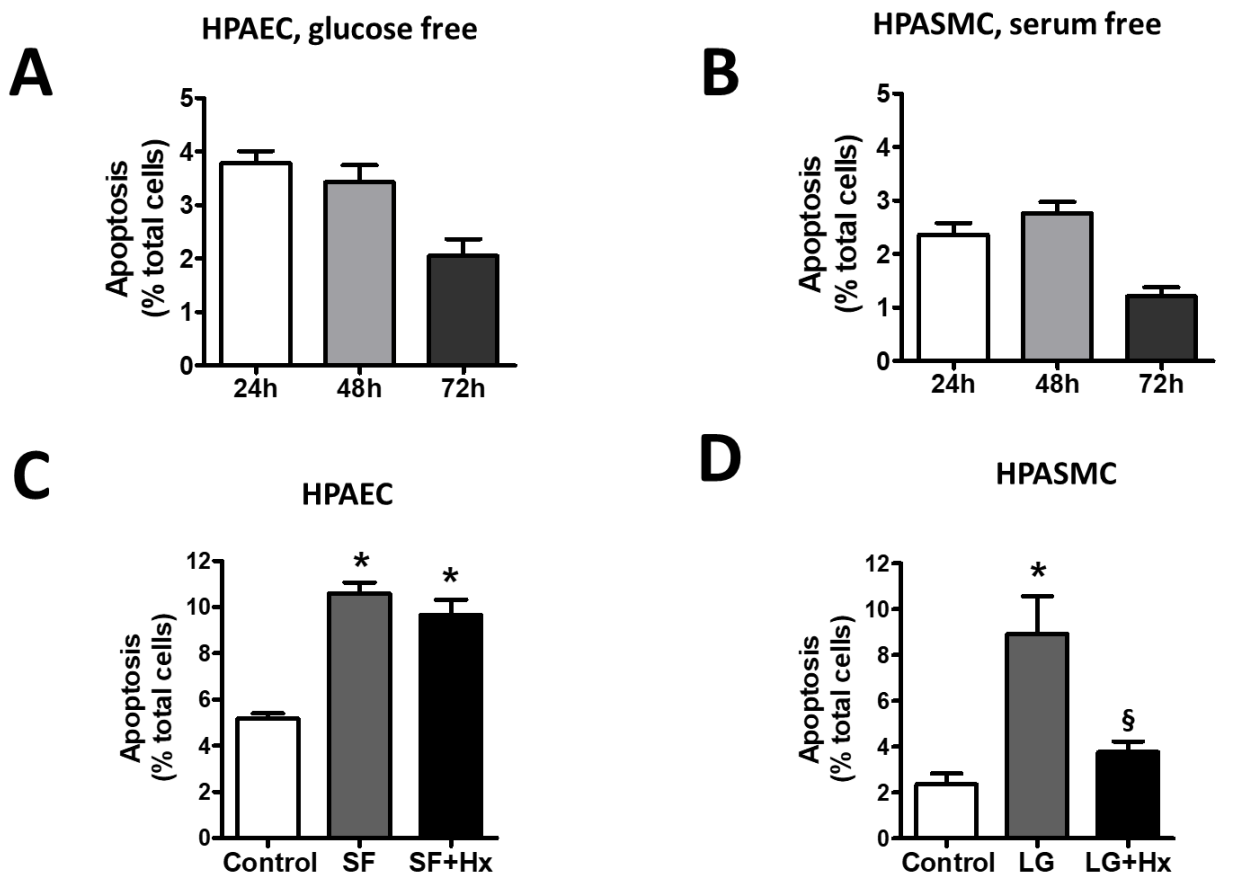

Figure S1. Validation of cell-specific conditions to induce apoptosis in HPAEC and HPASMC. Incubation of HPAEC in glucose free media (**A**, N=4) or HPASMC in serum free media (**B**, N=6) did not induce an increase in apoptotic cell death. In contrast, culturing HPAEC in serum free media (**C**, N=3) or HPASMC in low glucose (**D**, N=3) stimulated cell apoptosis, although exposure of cells to hypoxia did not further increase (**C**) or even attenuated (**D**) apoptotic cell death. Data presented as Mean±SEM, *P<0.05 vs. Control cells; §P<0.05 vs. non-hypoxic low glucose HPASMC. SF – serum free media; LG – log glucose (1 g/L) media; Hx - hypoxia (1% of O_2 for 48 hours).

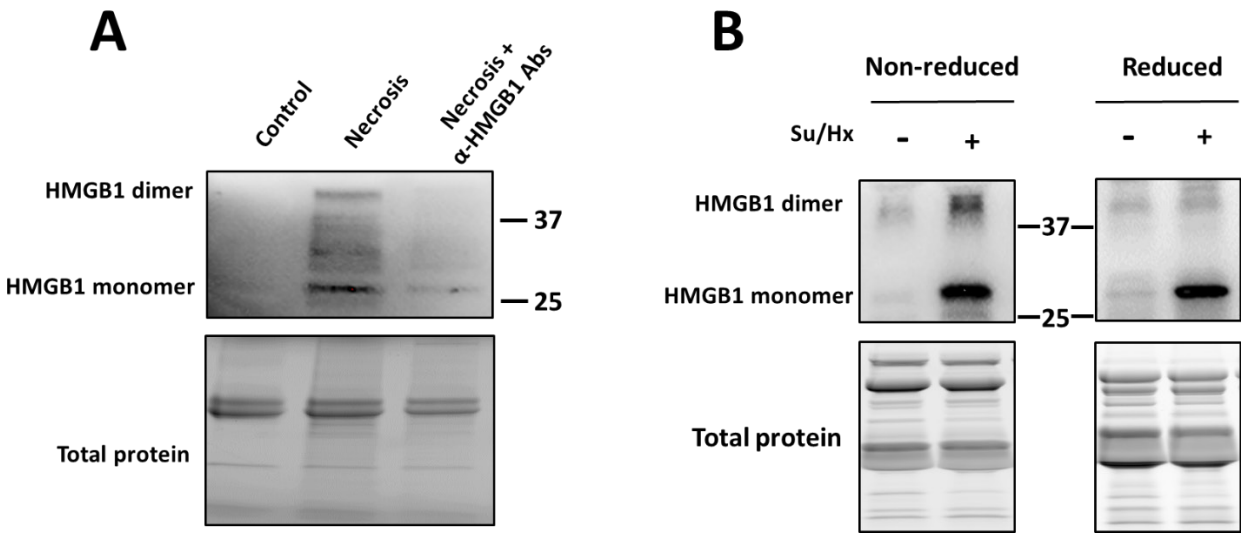

Figure S2. Validation of HMGB1 monomeric and dimeric signal in conditioned media and rat plasma. To confirm that signal that corresponds to HMGB1 monomers and dimers belong to HMGB1, we depleted HMGB1 in the conditioned media collected from necrotic HPASMC by incubation the media with HMGB1 neutralizing antibody followed by capturing of HMGB1-antibody complex with Protein A/G beads. The supernatant collected after beads removal contained almost no HMGB1 (either monomeric or dimeric) compared to the original necrotic conditioned media (**A**). The plasma samples collected from Control or PAH male rats were incubated with a sample buffer that contained or not the reducing agent, mercaptoethanol. The reducing conditions attenuated the signal from HMGB1 dimer (**B**).

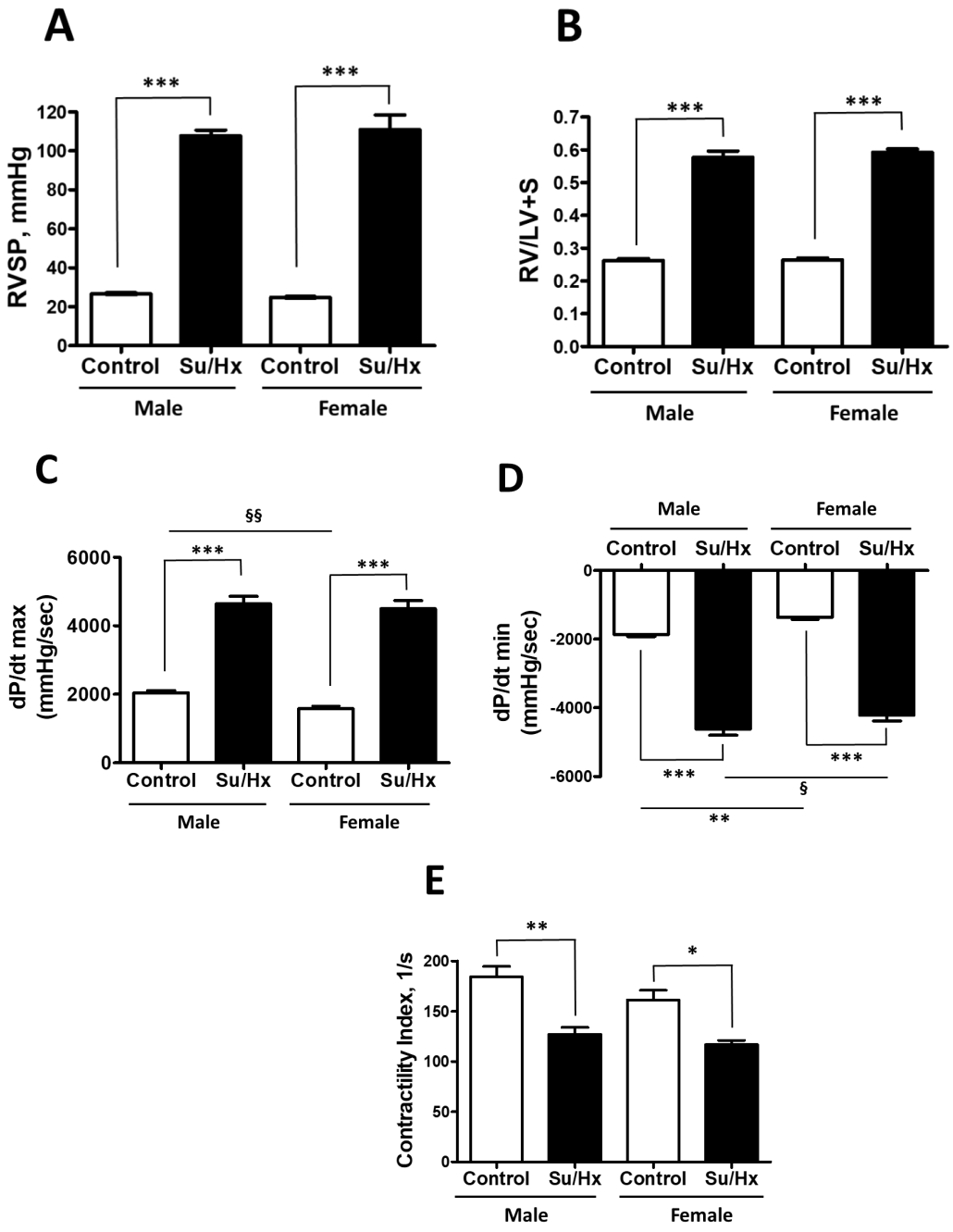

Figure S3. Hemodynamic changes in male and female rats with advanced PAH. Right ventricular (RV) systolic pressure (RVSP, **A**)**,** RV hypertrophy (**B**) and RV contractility assessed by either dP/dtmax (**C**) or by contractility index (**E**) were found to be comparable in male and female rats with an advance form of PAH induced by SU5416, 50 mg/kg s.c. followed by exposure to hypoxia (10% ± 0.5% of O_2) for 3 weeks and normoxia for another 2 weeks (5 weeks total) as previously published[25, 27]. RV dP/dt as a measure of RV relaxation (**D**) preserved the initial sex difference. Data presented as Mean±SEM, *P<0.05, **P<0.01, ***P<0.001 vs. sex-matched Controls; §P<0.05, vs. Male Su/Hx. N=8 in Controls, N=4 in male Su/Hx and N=5 in female Su/Hx.

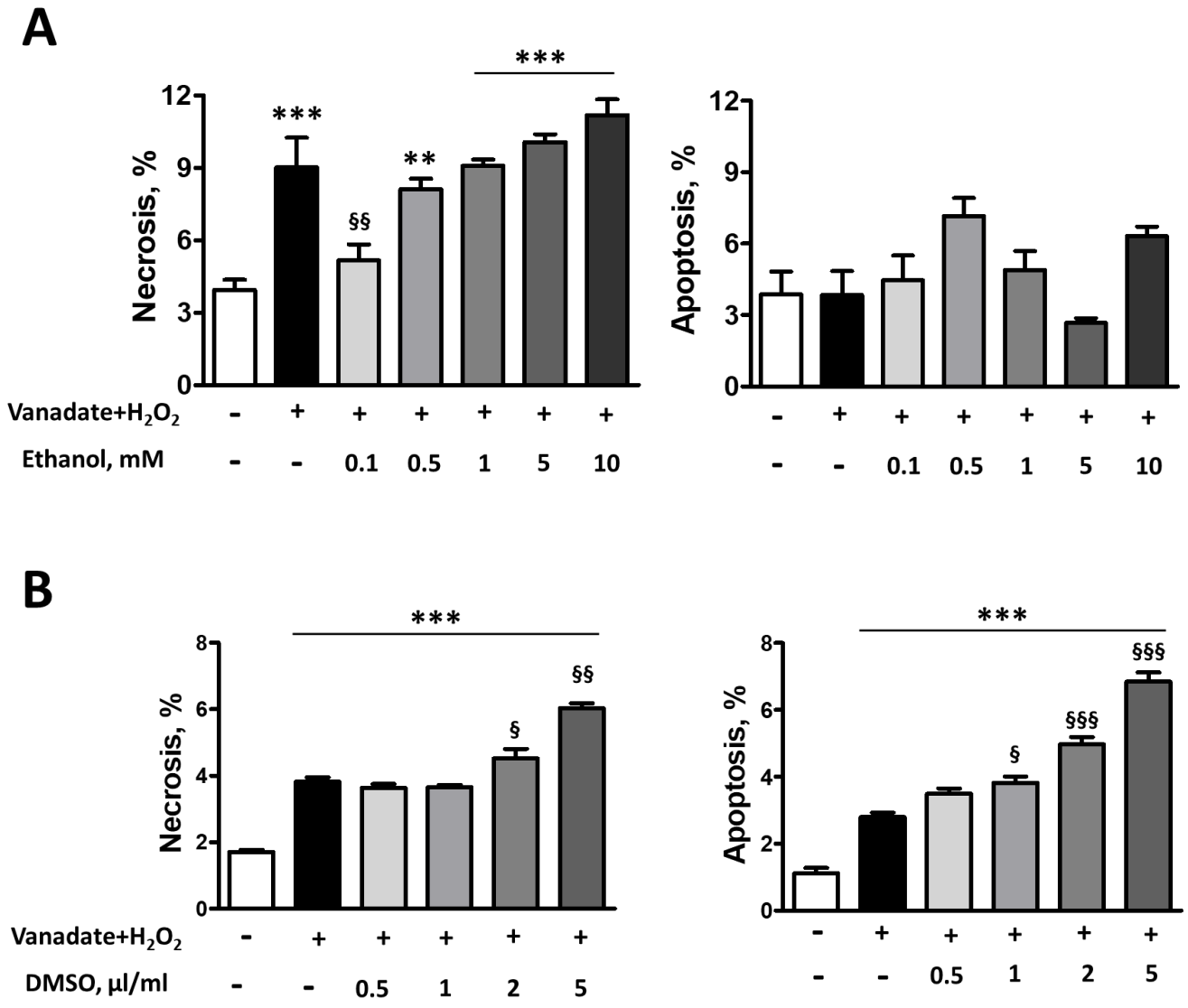

Figure S4. Effects of vehicles on the levels of PASMC apoptosis and necrosis. The dose of the EtOH (0.1 mM) used as a vehicle for NX-5 was found to effectively protect RPASMC from necrotic cell death without altering the apoptotic levels (**A,** N=4-5). In contrast, the dose of DMSO (1 µl/ml) used as a vehicle for Z-VAD and NCST did not alter the level of necrosis but induced a mild change in the cell apoptosis (**B**, N=6). Data presented as Mean±SEM, **P<0.01, ***P<0.001 vs. untreated PASMC; §P<0.05, §§P<0.01, §§§P<0.001 vs. Vanadate+H_2O_2 treated cells.

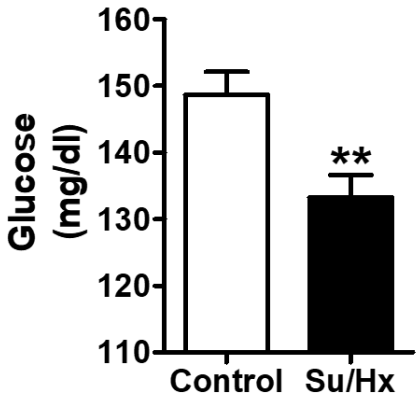

Figure S5. Decreased plasma glucose in PAH rats. The plasma levels of glucose in PAH rats (after two weeks in hypoxia as published[25]) were found to be significantly decreased compared to control animals. Data presented as Mean±SEM, **P<0.01 vs. Control rats. N=6 in all groups.