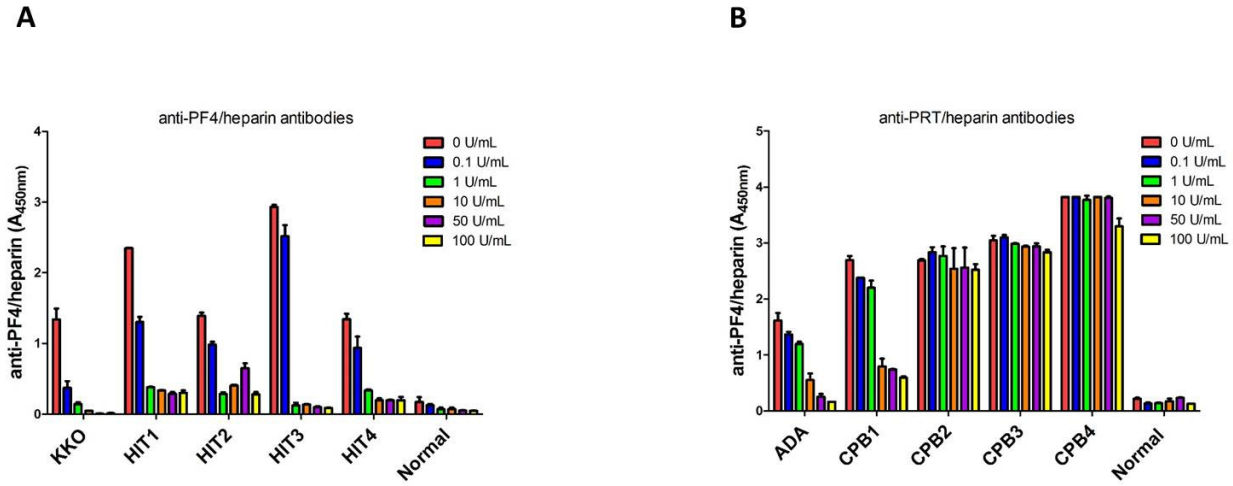
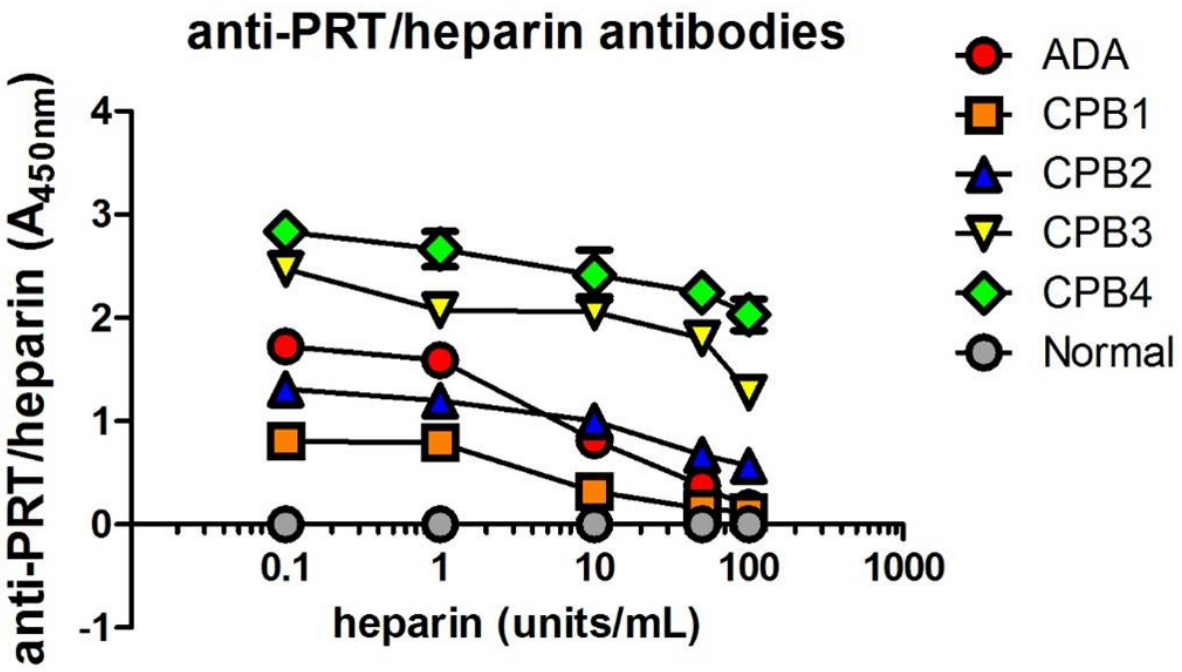
Supplemental Figure 1



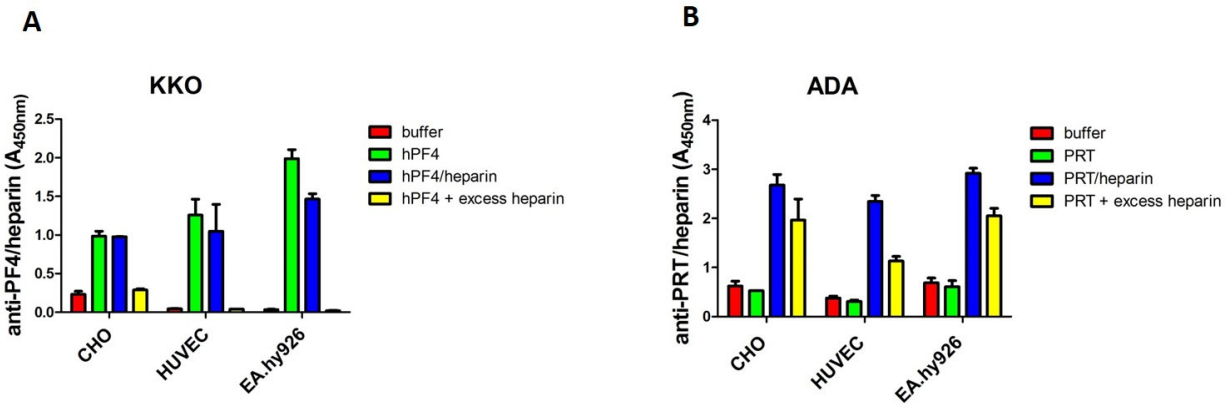
Supplemental Figure 1: Heparin-dependent reactivity of monoclonal and polyclonal antibodies to PF4/heparin and PRT/heparin complexes. In order to demonstrate binding of anti-PF4/heparin **(Panel A)** and anti-PRT/heparin antibodies **(Panel B)** to PF4/heparin and PRT/heparin in the absence of excess heparin (0 units/mL), data presented in Figure 2 is depicted in column graph format. All data shown are representative of 3 independent determinations.

Supplemental Figure 2



Supplemental Figure 2: Heparin-dependent reactivity of ADA and patient-derived PRT/heparin antibodies to PRT/heparin in the presence of excess heparin at a higher dilution. As described in Figure 2B, ADA 100 ng/mL, patient-derived PRT/heparin antibodies (CPB 1-4) or normal plasma were incubated in microtiter wells coated with PRT/heparin complexes either in buffer or buffer supplemented with increasing concentrations of unfractionated heparin (0.1-100 U/mL). CPB samples and normal plasma were diluted to 1:2000. The pattern of antibody binding at 1:2000 was similar to that seen in Figure 2B at a 1:500 dilution. Mean absorbance of duplicate wells is shown on the y-axis. All data shown are representative of 3 independent determinations.

Supplemental Figure 3



Supplemental Figure 3: Binding of KKO and ADA to cell-surface GAGs. (A) Binding of KKO to cell-surface GAGs. Fixed cells (CHO, HUVEC, or EA.hy926 cells) were incubated with buffer, human PF4 (hPF4, 10 ug/mL), or hPF4/heparin complexes (10 ug/mL : 0.4 units/mL) for one hour at room temperature. After washing, microtiter wells were incubated with KKO 50 ng/mL in buffer or in buffer containing excess heparin (100 units/mL). **(B) Binding of ADA to cell-surface GAGs.** As described in (A), fixed cells were incubated with buffer, PRT 31 ug/mL, or PRT/heparin complexes (31 ug/mL : 4 units/mL) followed by ADA 1.25 ug/mL in buffer or in buffer containing excess heparin (100 units/mL). All data shown are representative of 3 independent determinations.

Supplemental Table 1: Clinical information

	Age	Sex	Heparin exposure during hospitalization	Relevant medical history	Complications during hospitalization	Prior cardiac surgery
HIT1	**60**	**M**	**CABG**	**none**	**Bilateral hand/digit ischemia, left lower extremity DVT, PE, cardiogenic shock**	**none**
HIT2	**58**	**M**	**cardiac catheterization, IABP placement**	**none**	**PE**	**none**
HIT3	**53**	**M**	**LVAD placement**	**ischemic cardiomyopathy**	**none**	**none**
HIT4	**58**	**F**	**LVAD placement**	**ischemic cardiomyopathy**	**none**	**none**
CPB1	**57**	**F**	**CABG**	**history of PVD requiring revascularization and stenting**	**no clinical follow-up available**	**none**
CPB2	**63**	**M**	**CABG**	**none**	**none**	**none**
CPB3	**55**	**F**	**CABG**	**none**	**none**	**none**
CPB4	**30**	**F**	**MV repair**	**none**	**no clinical follow-up available**	**none**

CABG, coronary artery bypass grafting; DVT, deep vein thrombosis; PE, pulmonary embolism; IABP, intra-aortic balloon pump; LVAD, left ventricular assist device; PVD, peripheral vascular disease; MV, mitral valve