

# **FcRn Rescues Recombinant Factor VIII Fc Fusion Protein from a VWF Independent FVIII Clearance Pathway in Mouse Hepatocytes**

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## **SUPPLEMENTAL MATERIAL AND METHODS**

### **Clodrosome mediated Kupffer cell depletion**

Kupffer cells and macrophages were depleted by a single intravenous dose of 10 mL/kg (50 mg/kg) clodrosomes, liposome encapsulated clodronate (Encapsula nanosciences LCC, Nashville, TN) and compared with control encapsomes, phosphate buffered saline loaded liposomes [1,2]. Mice were dosed 24 hours before the start of pharmacokinetic studies. Blood macrophage depletion was confirmed by flow cytometry of leucocytes following erythrocyte lysis. Tissue macrophage cell depletion was confirmed by qPCR analysis of RNA isolated from spleen, liver and lung. Kupffer cell depletion was quantified by immunohistochemical analysis of the surface area decrease in formalin liver sections stained for iba-1 or F4/80 (not shown).

### **Supplemental References**

1. Yamamoto T, Naito M, Moriyama H, Umezu H, Matsuo H, Kiwada H, et al. Repopulation of murine Kupffer cells after intravenous administration of liposome-encapsulated dichloromethylene diphosphonate. *Am J Pathol.* 1996 Oct;149(4):1271–86.
2. van Rooijen N, Hendrikx E. Liposomes for specific depletion of macrophages from organs and tissues. *Methods Mol Biol.* 2010;605:189–203.