Supplementary Figure 1
Supplementary Figure 1. Expression and purification of SFRP1 and its CRD and NTR domains. (a) The conditioned media from MDCK/control (C) or MDCK/SFRP1 individual clones (1-8) were tested for the presence of secreted SFRP1 by immunoblot with anti-myc antibody. Clones 2, 7 and 8 secreted the highest yield of SFRP1. (b) Silver staining (lanes 1-3) and immunoblot (lanes 4, 5) with anti-myc antibody of representative fractions eluted as described (see Supplementary Methods online) from control (lanes 2, 4) or clone 2 (lanes 3, 5) concentrated conditioned media. Eluted fractions were separated on 10% SDS-PAGE. (c) Schematic diagram depicting the entire SFRP1 protein and the constructs containing the cystein rich domain (CRD) or the Netrin domain (NTR). Grey boxes correspond to the secretion signal peptide (SP); blue to the CRD and red to the NTR. Numbers indicate amino acid residues in SFRP1 sequence at boundaries of recombinant peptides. (d) The panel shows an anti-myc immunoblot to detect SFRP1 or its CRD and NTR derivatives in the conditioned medium from isolated clones of stably transfected MDCK. As already reported for similar constructs\cite{29}, both SFRP1 derivatives were detected in the culture media, at levels similar to those of the entire molecule.