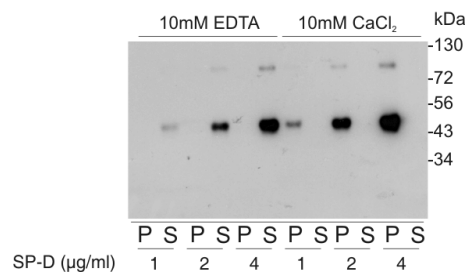


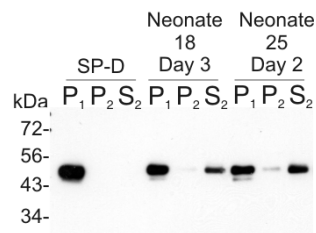
Supplementary Figure 1) An assay for SP-D lectin activity: Western blot of pellet (P) and supernatant (S) fractions from zymosan binding assays reveals binding of increasing concentrations of native SP-D to zymosan is inhibited by calcium chelation with EDTA. A major band migrating between 43 and 56kDa corresponds to the expected molecular weight for fully reduced SP-D monomeric subunits. Minor bands mobilising at approximately 95kDa correspond to partially unreduced SP-D dimers.

Supplementary Figure 1



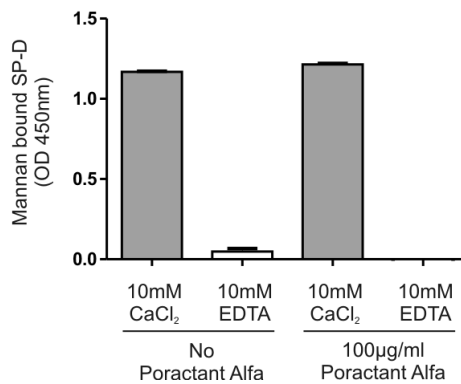
Supplementary Figure 2) Zymosan saturation does not explain the restricted SP-D lectin activity: Following one round of zymosan binding, supernatants from experiments carried out with native SP-D (lanes 1-3) or BALF from two neonates (lanes 4-6 & 7-9) were subjected to a second binding step. Following Western blot under reducing conditions, SP-D found in the supernatant of BALF from the first round showed negligible binding to zymosan in a second round (P₂) and was mainly found in the supernatant from the second round (S₂). All native SP-D bound the zymosan pellet in the first round (P₁). Blots are representative of three independent experiments.

Supplementary Figure 2



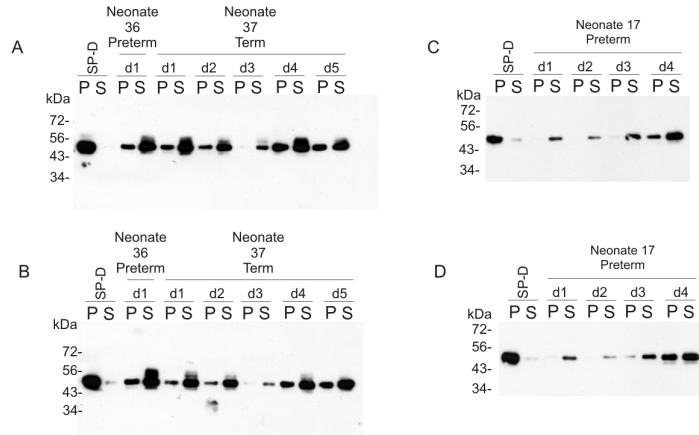
Supplementary Figure 3) Exogenous surfactant does not inhibit native SP-D lectin activity: Mannan (50µg/ml) was coated onto a 96-well plate and used as a substrate to measure the impact of the exogenous surfactant, Poractant Alfa, on SP-D lectin activity. Native SP-D bound equally well to mannan in the presence or absence surfactant, however binding was almost completely abrogated by 10mM EDTA. Data represents the mean of triplicate values from one of three independent experiments.

Supplementary Figure 3



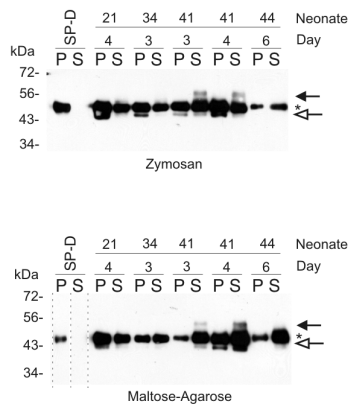
Supplementary Figure 4) Analysis of SP-D binding activity in neonatal BALF: Term and preterm BALFs were assessed in duplicate independent zymosan binding assays. Following SDS-PAGE and Western blot for SP-D, native SP-D positive control was predominantly found in the bound pellet (P) fraction. Occasionally a minor proportion was observed in the non-bound supernatant fraction (S). In the case of BALFs, a large proportion of SP-D was consistently found in the non-bound supernatant fraction. These findings were reproducible across duplicate independent experiments undertaken for all BALFs and representative examples are shown here (A&B, C&D).

Supplementary Figure 4



Supplementary Figure 5) A high proportion of neonatal BALF SP-D also fails to bind maltose-agarose: Neonatal BALF or native SP-D was tested in parallel for binding to zymosan (upper panel) or maltose-agarose (lower panel). SDS-PAGE followed by Western blot of bound pellet fractions (P) and non-bound supernatant (S) fractions revealed that a substantial proportion of SP-D in neonatal BALF failed to bind to maltose-agarose. In some cases (neonate 41, day 3 & 4) less material appeared capable of binding to maltose-agarose than zymosan. Bands migrating 3-5kDa above (closed arrows) and 1-2 kDa below (open arrows) the main reduced monomeric subunit of SP-D (asterisk), were visible in the maltose-agarose binding assay as well as the zymosan binding assays. The higher molecular weight band was found exclusively in the non-bound supernatant fraction in both zymosan and maltose-agarose binding assays. Data are representative of 4 independent experiments assessing 15 BALF samples. Dotted lines in the maltose-agarose blot indicate where lanes have been moved within the gel image to facilitate comparison with the zymosan blot above due to a discrepancy in the original loading of SDS-PAGE lanes.

Supplementary Figure 5



Supplementary Figure 6) SP-D which fails to bind zymosan does not exhibit substantial additional binding activity towards mannan: Neonatal BALF containing an approximately equal proportion of bound and non-bound SP-D in zymosan binding assays (panel B) was selected for input into a solid phase mannan binding assay in 96 well plates. Input material for the binding assays were: 1) Native SP-D (NhSP-D) (1 μ g/ml), 2) BALF (adjusted to achieve 1 μ g/ml ELISA equivalent each of zymosan-binding and non-binding SP-D) or 3) supernatant (Supt.) aspirated following a zymosan binding assay (adjusted to achieve 1 μ g/ml ELISA equivalent of SP-D). All input materials were diluted either in TBS-Tween plus 10mM CaCl₂ (black bars), 10mM CaCl₂ plus 100mM D-maltose (grey bars) or 10mM EDTA (open bars). After a 90 minute binding step, bound SP-D was detected by sequential incubation with goat anti-SP-D and donkey anti-goat-HRPO, followed by the addition of TMB substrate. Presented data are expressed as mannan bound SP-D minus background binding to wells coated with PBS alone.

Supplementary Figure 6

