

Functional repertoire of EV-associated miRNA profiles after lipoprotein depletion via ultracentrifugation and size exclusion chromatography from autologous blood products

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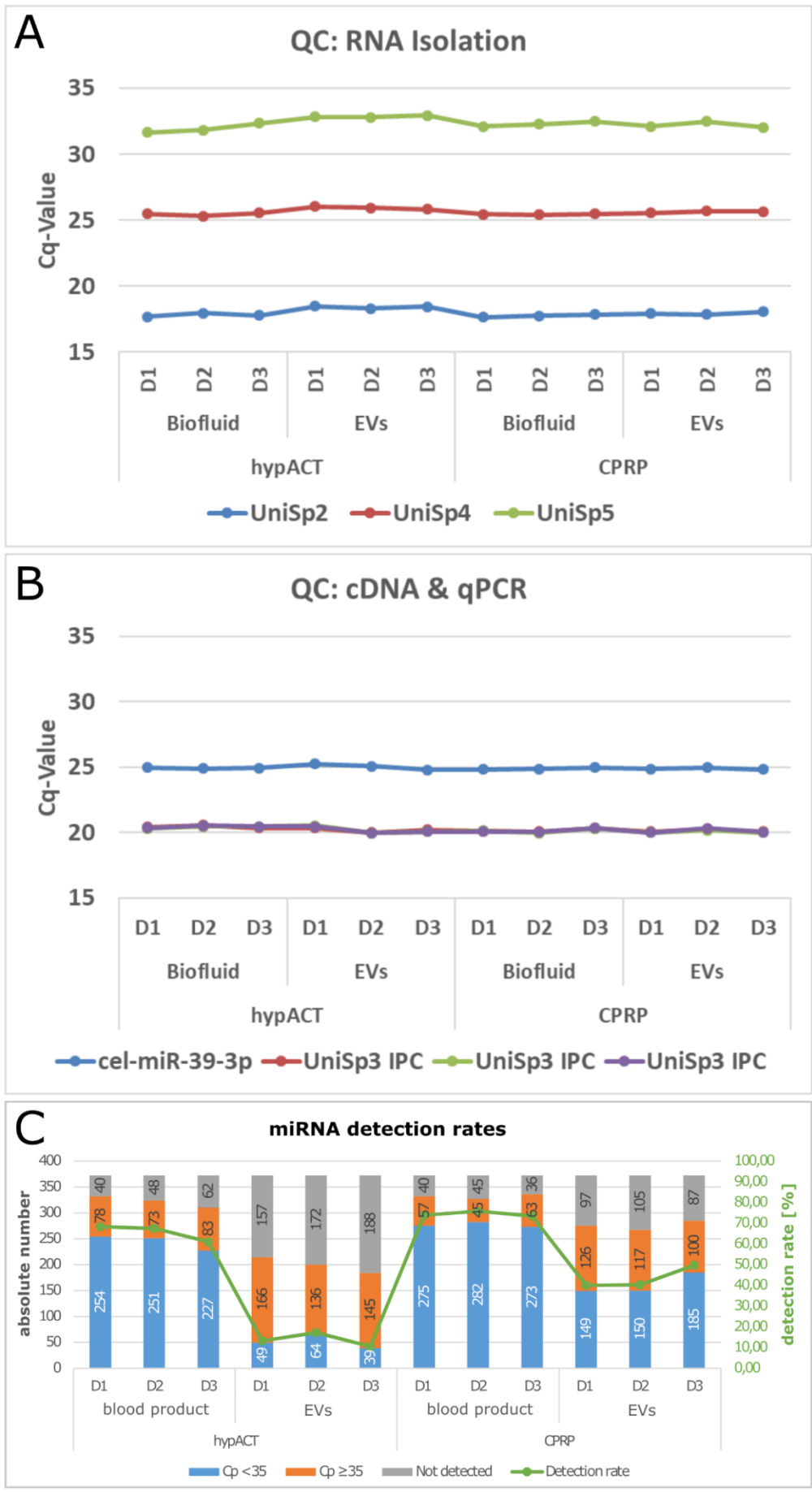


Fig S1. Quality control of RT-qPCR-based miRNA quantification and miRNA detection rates (A) RNA extraction controls, (B) reverse transcription controls. (C) Number of miRNA species identified per sample.

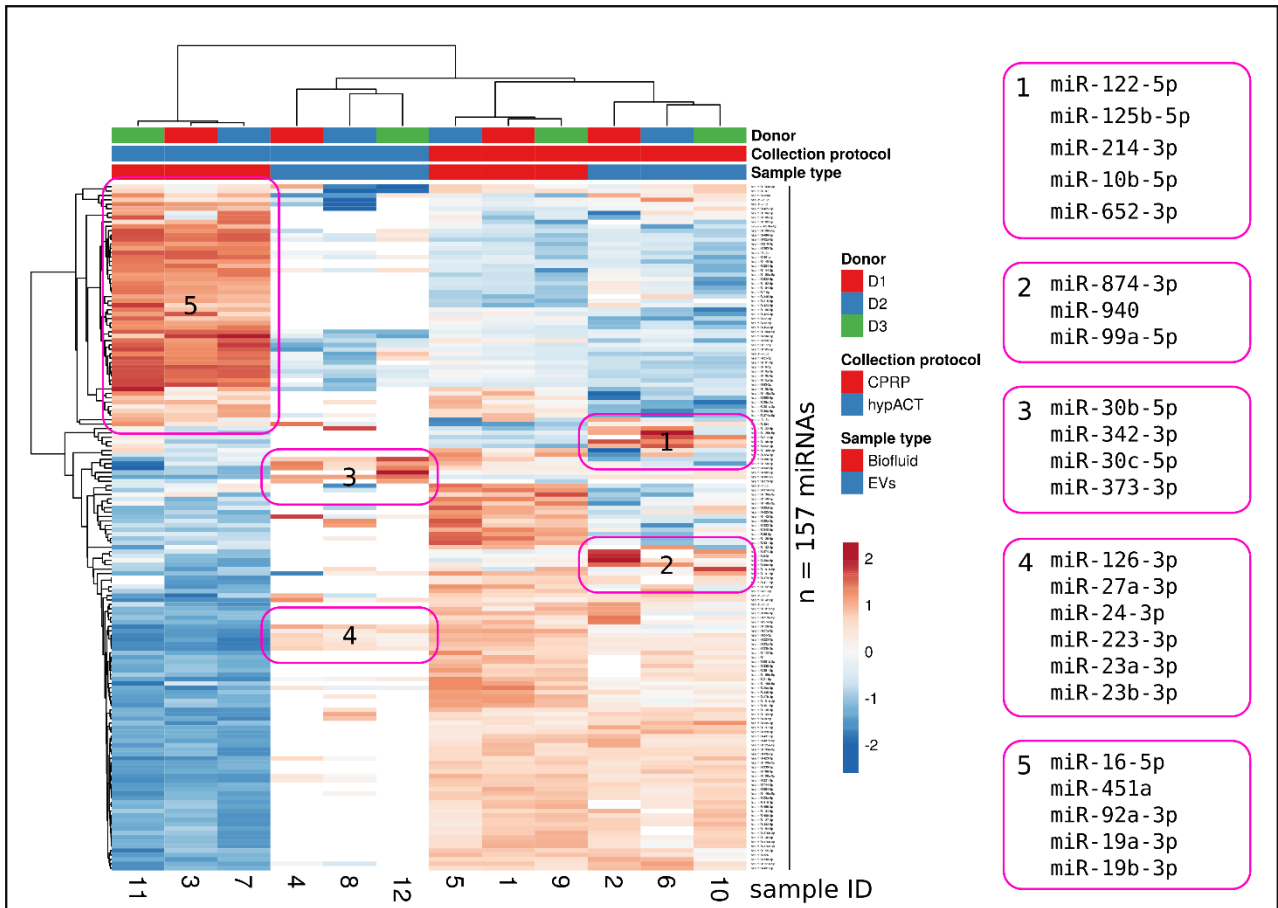


Fig S2. Heat map of ΔC_p values, normalised to let-7a. Hierarchical clustering analysis was performed via ClustVis⁷⁵ using the default settings, i.e. singular value decomposition with imputation, pearson correlation as the distance metric, and average distance for clustering.

Supplementary data 1 – PHP implementation of Savitzky-Golay algorithm

```
/*
 *
 * Perform size distribution smooting using the Savitzky-Golay polynomial approach
 *
 * @ref statistics4u.info/fundstat_eng/cc_filter_savgolay.html
 * @ref statistics4u.info/fundstat_germ/cc_savgol_coeff.html
 *
 * @param array $a - size distribution as list of concentrations
 * @ $neighbours int $neighbours - chosen number of neighbours of target value
 *
 * @return array $smoothed_a - smoothed size distribution
 */
function savitzky_golay_smooth ($a,$neighbours) {

    $neighbours = intval($neighbours);

    if ( is_numeric($neighbours) && $neighbours > 1 && $neighbours < 13 ) {

        //backup
        $smoothed_a = $a;
        //list of coefficients indexed by neighbour parameter
        //np is the number of values used for smoothing
        $coefficients = array(
            2 => array(-3,12,17,12,-3), //np=5
            3 => array(-2,3,6,7,6,3,-2), //np=7
            4 => array(-21,14,39,54,59,54,39,14,21), //np=9
            5 => array(-36,9,44,69,84,89,84,69,44,9,-36), //np=11
            6 => array(-11,0,9,16,21,24,25,24,21,16,9,0,-11), //np=13
            7 => array(-78,-13,42,87,122,147,162,167,162,147,122,87,42,-13,-78), //np=15
            8 => array(-21,-6,7,18,27,34,39,42,43,42,39,34,27,18,7,-6,-21), //np=17
            9 => array(-136,-51,24,89,144,189,224,249,264,269,264,249,224,189,144,89,24,-51,-136), //np=19
            10 => array(-171,-76,9,84,149,204,249,284,309,324,329,324,309,284,249,204,149,84,9,-76,-171), //np=21
            11 => array(-42,-21,-2,15,30,43,54,63,70,75,78,79,78,75,70,63,54,43,30,15,-2,-21,-42), //np=23
            12 => array(-253,-138,-33,62,147,222,287,343,387,422,447,462,467,462,447,422,387,343,287,222,147,62,-33,-138,-253) //np=25
        );
        //list of normalisation factors indexed by neighbour parameter
        $h = array(
            2 => 35,
            3 => 21,
            4 => 231,
            5 => 429,
            6 => 143,
            7 => 1105,
            8 => 323,
            9 => 2261,
            10 => 3059,
            11 => 805,
            12 => 5175
        );

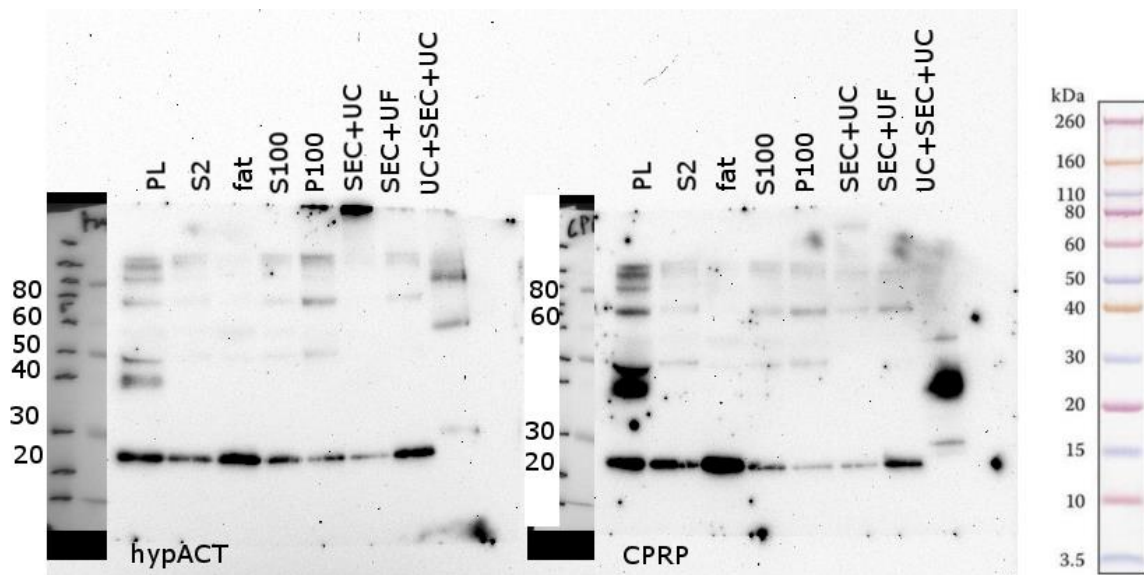
        //define position range
        $positions = range(-$neighbours,$neighbours);

        //perform smoothing
        for ($i=$neighbours;$i<count($a)-$neighbours;$i++) {
            //calculate
            for ($y=0,$j=0;$j<count($coefficients[$neighbours]);$j++) {
                $y += $coefficients[$neighbours][$j]*$a[$i+$positions[$j]];
            }
            //normalize
            $y = $y/$h[$neighbours];
            //set negation value to 0, as there can't be negative particle concentration
            if ( $y < 0 ) {
                $smoothed_a[$i] = 0;
            } else {
                $smoothed_a[$i] = $y;
            }
        }
        } else {
            die("Wrong neighbours parameter for Savitzky-Golay smoothing");
        }
        return $smoothed_a;
    }
}
```

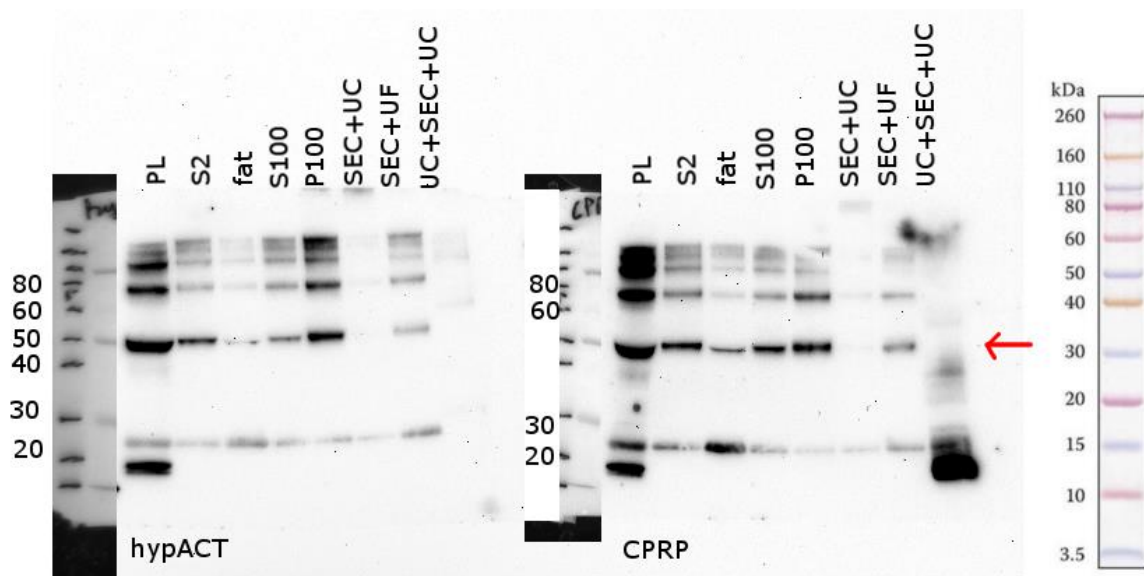
Supplementary data 2 – Full Western Blot scans

Blots in Fig 3A

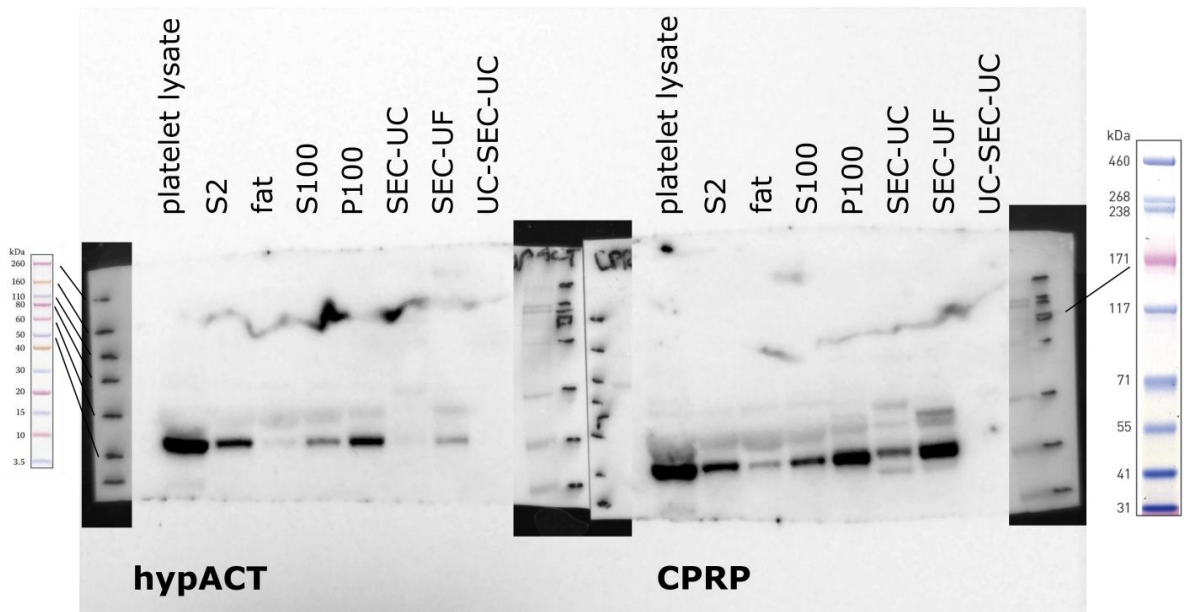
ApoA1, 10 μg per lane, except PL (20 μg)
10% Bis/Tris SDS-PAGE, non-reducing



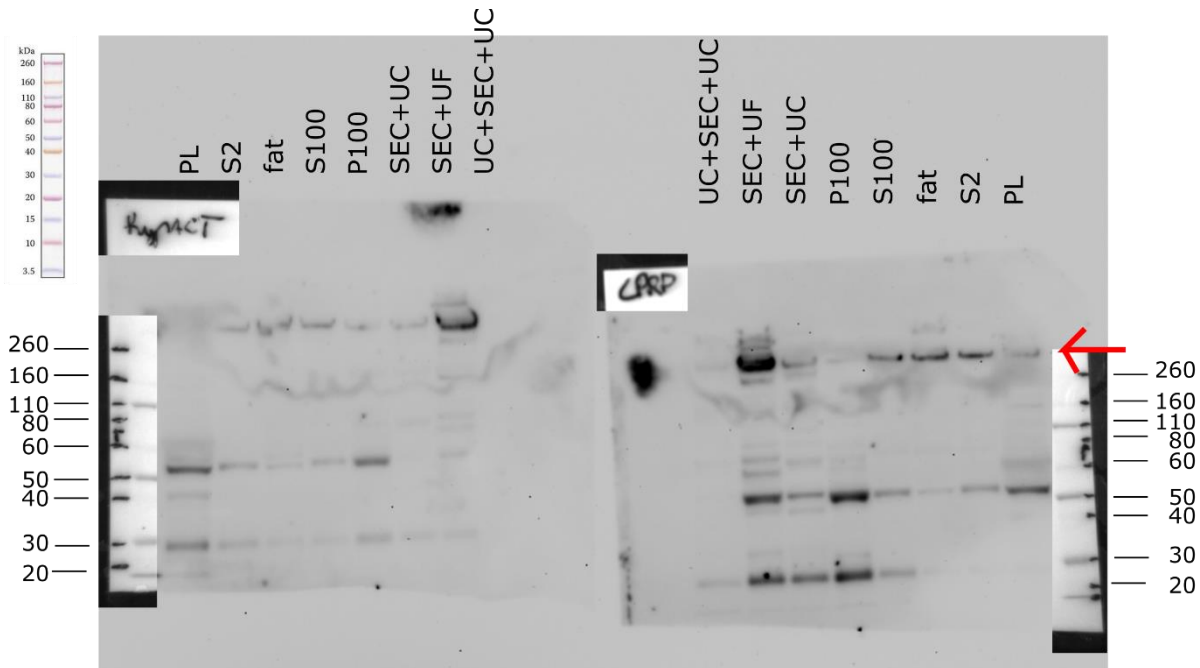
CD9, 10 μg per lane, except PL (20 μg)
10% Bis/Tris SDS-PAGE, non-reducing



Alix, 10 μg per lane, except PL (20 μg)
4-12% Bis/Tris SDS-PAGE, reducing

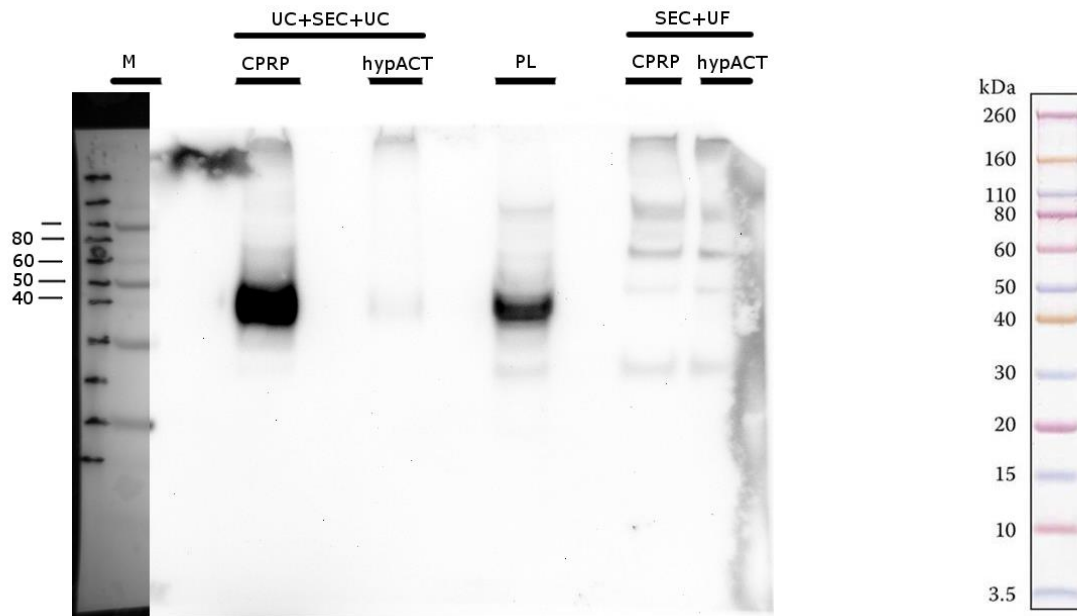


ApoB100, 10 μg per lane, except PL (20 μg)
4-12% Bis/Tris SDS-PAGE, reducing



Blots in Fig 3B

CD63, 80 μ g per lane, except PL (20 μ g)
 10% Bis/Tris SDS-PAGE, non-reducing



CD9, 80 μ g per lane, except PL (20 μ g); reprobed after detecting CD63 and washing
 10% Bis/Tris SDS-PAGE, non-reducing

